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THE DISCOVERY OF *HISTOPLASMA CAPSULATUM* IN CONNECTICUT SOIL INCIDENTAL TO THE INVESTIGATION OF A CASE OF FELINE CRYPTOCOCCOSIS

by

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(with two figs.)

(19.VIII.1960)

The rarity of recorded cases of cryptococcosis in cats suggests that this disease is infrequently encountered in this species. A review of the literature reveals only six such infections. HOLZWORTH (1952) and HOLZWORTH & COFFIN (1953) described two cases in cats in the Unites States. In each instance the animals involved presented signs of a chronic upper respiratory disorder. In both cases the diagnosis was made by histological examination which revealed infiltration of tissues by encapsulated budding yeast-like cells. Three additional feline cases were cited in the United States by McGRATH (1954). In these cats, the organism was found to have invaded the central nervous system. In 1957, YAMAMOTO, ISHIDA & SATO (1957) described a case of pulmonary cryptococcosis in a Japanese cat. They recovered *Cryptococcus neoformans* from a granulomatous lesion in the right lung and demonstrated by histological studies the presence of the organism in the affected tissues.

In addition to cats, cryptococcosis has been diagnosed in a diversity of mammalian species, including man (LITTMAN & ZIMMERMAN, 1956, AJELLO, 1958). However, there is no evidence that the disease is naturally transmitted from one host to another. Infection is contracted by exposure to a reservoir in nature. This fact has been established by the repeated isolations of pathogenic strains of *Cryptococcus neoformans* from soil and pigeon droppings and pigeon nests (EMMONS 1951, 1955, 1960, YAMAMOTO et al. 1957, KAO &

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Schwarz, 1957, Littman & Schneierson, 1959). The respiratory tract is regarded by most workers as the usual portal of entry of the causal agent. Evidence for this view is twofold: first, the organism has been isolated from the air (Lodder & Kreger-Van Rij, 1952). Second, many human patients with cryptococcosis have a primary focus of infection in the lungs, an observation which suggests inhalation. However, there is evidence which indicates that *C. neoformans* may occasionally gain entrance into the body through the skin or mucous membranes (Littman et al. 1956).

In the present paper, the seventh case of cryptococcosis in a cat is recorded. In this instance a limited study was carried out to find the possible source of infection. While this epizootiological investigation was unsuccessful in terms of locating such a source, it resulted in the unexpected isolation of *Histoplasma capsulatum* from soil in an area where this fungus had not been recovered previously. With the conventional mycologic techniques employed two months were required to make this isolation. This time factor led us to investigate the possibility of applying the fluorescent antibody technique for the rapid detection of *H. capsulatum* in soil. The preliminary findings of that study are also reported.

CASE HISTORY

On January 25, 1959, a five-year-old spayed female Maltese short hair cat was brought to one of the authors (D. D. B.). The animal at that time showed two tumor-like masses on the underside of the neck, near the throat. On January 27, 1959, the enlargements were removed surgically. Ten days later a mass the size of a hen's egg developed at the site of one of the original lesions. Also, at that time, a moist skin lesion was noted on the flank. Portions of the skin lesion and the tumorous mass were removed and fixed in 10 % formalin for histological examination. Cultures were not prepared. The animal was then destroyed and cremated.

Histological study of hematoxylin and eosin stained sections showed proliferation within the tissues of large numbers of encapsulated, budding yeast-like cells with a morphology characteristic of *C. neoformans* (Fig. 1). The inflammatory response to invasion by the organism appeared to be minimal. On the basis of the histological examination, a diagnosis of cryptococcosis was made.

EPIZOOTIOLOGICAL INVESTIGATION

In view of the apparent infrequent occurrence of feline cryptococcosis, an investigation was initiated to find the possible source of the infection. The owner was queried with respect to exposure of the cat to soil or avian habitats. This individual, who resided in Stratford, Connecticut, stated that due to inclement weather, the animal had been kept indoors since early winter. Therefore, the only direct soil contact had been with a recently purchased potted poinsettia plant. During the warm months of the year, the cat had been permitted to go out-of-doors. On the premises, near the family dwelling, the owner had a bird house and bird bath. Few pigeons were reported to have frequented the area.

Samples of soil from the potted poinsettia, from the vicinity of the bird house and bath and from two other sites on the grounds were collected in four-ounce sterile screw cap jars for mycological examination.

Further investigation disclosed that the owner had purchased the potted poinsettia from a local florist who had acquired the plant from a wholesale greenhouse company in North Haven, Connecticut. The owner of the wholesale establishment reported that the soil for potting the poinsettia had been obtained from a large mound of earth dumped near the greenhouse. The soil in the mound had in turn been collected in an old orchard adjacent to the greenhouse. Cows had grazed in the orchard prior to removal of the soil. In addition, the wholesale florist recalled that chickens had been kept, in the past, near the orchard. Eight soil samples were collected from the mound for mycological study.

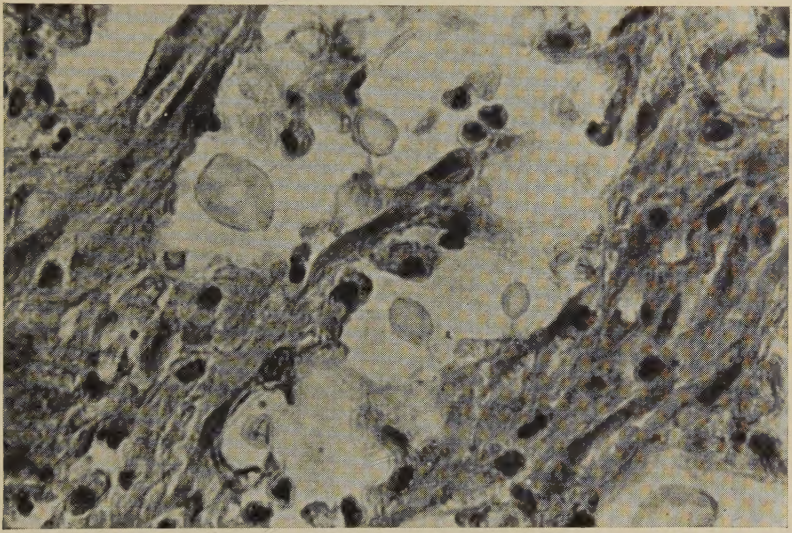

Fig. 1. Section of tumor-like lesion of the skin, showing heavily encapsulated yeast-like cells. Several leucocytes are intermingled with the fungus cells. ×475

MYCOLOGICAL STUDIES OF SOILS

All soil samples collected during the course of this study were examined for the presence of *C. neoformans* and other pathogenic fungi. The procedure employed was the one described in detail by

Ajello & Runyon (1953). Essentially this method consists of the intraperitoneal inoculation into each of four white mice with one ml of a physiological saline suspension of soil to be tested. After six weeks the mice are sacrificed and portions of the liver and spleen are cultured for the presence of pathogenic fungi.

None of the samples collected yielded *C. neoformans*. However, *H. capsulatum* was recovered from the potted poinsettia soil. This fungus was not isolated from the other samples.

FLUORESCENT ANTIBODY STUDIES OF SOIL

On the basis of the favorable reports of a number of investigators (Thomason, Moody & Goldman, 1956, Moody, Ellis & Updyke, 1958, White & Ristic, 1959, Goldwasser & Kissling, 1958) it would appear that one of the more important applications of the fluorescent antibody technique is the rapid identification of pathogenic organisms in clinical materials and in cultures. Such reports led us to investigate the potential value of this immuno-chemical staining procedure for the rapid detection of *H. capsulatum* in soil samples.

Fluorescein-labeled rabbit anti-*H. capsulatum* globulins were used as a stain. The procedures employed for preparation of such conjugates are described in detail in another publication (Kaplan & Ivens, 1960). Fluorescein-labeled normal rabbit globulins were also employed as a control.

Smears for staining with the conjugates were prepared from the sediment of centrifuged (10,000 rpm) saline suspensions of soil samples. Such smears were allowed to air-dry, then fixed by heat. The staining process was carried out by covering the preparations with labeled globulins and permitting them to stand for 45 minutes at 37° C. Following this treatment, the stained smears were rinsed in saline for 10 minutes, then in distilled water for five minutes and carefully blotted dry. A drop of mounting fluid containing 9 parts C.P. glycerine and one part phosphate buffered saline (pH 7.2) was placed on each preparation and covered with a cover glass.

A high-pressure mercury vapor lamp (Osram HBO-200) mounted on a Reichert Lux U. V. assembly was employed as the light source for visualization. The microscope used was the Reichert Biozet fitted with a cardioid darkfield condenser. A No. 5113 Corning Glass barrier filter and a Wratten 2A ocular filter were employed for the examination of stained preparations.

Examination of smears from the potted poinsettia plant soil that had been treated with the labeled immune globulins revealed several brightly staining round to oval forms (Fig. 2). These elements varied in size from 1.5 to 3.5 microns in diameter and their morphology was consistent with that of the microconidia of *H. capsulatum*. No tuberculate spores or mycelial filaments were detected. Careful examination of similar soil smears treated with labeled normal globulins did not reveal the presence of such stained forms.

Five additional soil samples that had previously been shown by the mouse inoculation test to contain *H. capsulatum* were also studied by the fluorescent antibody technique. Two of the samples had been gathered from a cave in Tanganyika, Africa, two from a village in Venezuela, and one from Lexington, Kentucky. Smears from all five samples showed similar fluorescent round to oval forms when stained with the immune conjugates. Similar smears that had been treated with normal conjugates were negative.

An investigation is currently underway to determine whether the fluorescent forms might be elements of other fungus species that possess antigens in common with *H. capsulatum*. For that study, the sediment of the centrifuged (10,000 rpm) saline suspension of poinsettia plant soil is streaked on Sabouraud dextrose agar plates. Colonies of micro-organisms are picked and transferred to tubes of Sabouraud dextrose agar. Smears of suspensions from such isolates are stained by the fluorescent method. To date, no cross-reactions have been observed. However, these studies are still in progress.

Fig. 2. Smear of soil stained with fluorescein labeled anti-*H. capsulatum* rabbit globulins. A single brightly staining form is visible in the center of the photograph. × 625

DISCUSSION

With the resurgence of interest in medical mycology there has been a marked increase, in recent years, in the number of recognized and recorded cases of cryptococcosis in man. Hitherto considered a rarity, this disease is responsible for approximately 10 % of the 300 to 400 fatal mycoses reported each year in humans in the United States (LITTMAN et al. 1956). A similar situation has, as yet, not been demonstrated in our cat population. As evidenced by the scarcity of documented cases, cryptococcosis appears to be a relatively rare disease of felines — one that is of little more than academic interest. However, the possibility does exist that the disease is more frequent among cats than the available data would indicate. The demonstrated high frequency of occurrence of *C. neoformans* in nature, together with the many opportunities that cats have for exposure to this agent would suggest that infections are not uncommon and many cases pass unrecognized. The true prevalence of feline cryptococcosis will only be revealed when veterinarians show greater awareness of the occurrence of this disease and carry out more extensive histological and cultural studies of cats in their practice.

Small scale skin test surveys to determine the prevalence of histoplasmin sensitivity among Connecticut residents have been carried out by several investigators. In one such study (LOOSLI, BEADENKOPF, RICE & SAVAGE, 1951) seven (6.8 %) of 103 individuals tested were found to be reactors. In another survey (MANOS, FEREBEE & KERSCHBAUM, 1956) 111 residents from counties in Connecticut were skin tested. The sensitivity prevalence among individuals who had resided in the northwest quadrant of the state was found to fall in the range of 2 to 10 % reactors. The sensitivity among residents of the other sections of Connecticut was reported to be less than 2 %. These findings suggested the existence of *H. capsulatum* in the environment of this state. The isolation of this fungusfrom Connecticut soil, unequivocally establishes its existence in that state. This finding, together with the results of the limited histoplasmin surveys would indicate the desirability of carrying out detailed epidemiologic studies in order to more accurately delineate endemic localities within the state. The results of such studies would serve to further alert physicians and veterinarians to the possible occurrence of histoplasmosis among their patients.

The preliminary findings of the study to evaluate the fluorescent antibody technique for the rapid detection of *H. capsulatum* in soils are most encouraging. Although the number of soils tested to date is small, it was possible to detect the stained spores without difficulty. In addition, there is, at present, no evidence that the observed fluorescent forms are the spores of heterologous organisms possessing antigens in common with *H. capsulatum*. It is, however, premature to draw any definite conclusions regarding the reliability and practicality of this immuno-chemical staining for the rapid detection of *H. capsulatum* in soil. Such conclusions must await the results of expanded evaluation studies which are now in progress. What is certain is that if the dependability of the test is unequivocally established, a valuable diagnostic tool for soil studies will be available to the medical mycologist, one which will allow a saving of approximately two months time over conventional *H. capsulatum* isolation procedures.

In a study conducted by Brown, Cook, Ney & Hatch (1950) to determine the affect of particle size upon the retention of particulate matter in the lungs of humans, it was found that elements larger than five microns rarely reach the alveoli. In line with these results, Cozad & Furcolow (1953) suggested that the small, smooth-walled microconidia of *H. capsulatum* must be the infectious elements of this organism. These workers reasoned that the large tuberculate spores, due to their size, would be retained in the upper air passages. If we can assume at this time that the observed fluorescent antibody stained fungal elements are indeed the microconidia of *H. capsulatum* then it would appear that the infectious form of this fungus has been demonstrated in soil.

SUMMARY

The seventh case of cryptococcosis in a cat is described. The animal involved was a five-year-old Maltese short hair that had lived in Connecticut all of its life. An investigation was carried out to discover the point source of this feline infection. This search was unsuccessful but it did result in the isolation of *H. capsulatum* for the first time from Connecticut soil.

In addition, the preliminary findings of a study to evaluate the use of the fluorescent antibody technique for the rapid detection of *H. capsulatum* in soil are reported. Round to oval forms measuring 1.5 to 3.5 microns in diameter were demonstrated in smears of the positive Connecticut soil that had been stained with fluorescein labeled anti-*H. capsulatum* globulins. The morphology of these elements was similar to that of the microconidia of *H. capsulatum*. Through the use of this immune conjugate five additional positive soil samples from other areas were also shown to contain these forms. None of these six soils showed stained elements when treated with normal conjugates. The implications of these findings are discussed.

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STUDIES ON BAKER'S YEASTS OF EAST PAKISTAN II.

by

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INTRODUCTION

AHMAD and his colleagues isolated yeasts from apples, raisins, toddy and dough in order to find a suitable strain which could be utilised for the manufacture of food yeast in East Pakistan. As the dough gave some promising yeasts (AHMAD & KHAN 1955), it was decided to isolate some more yeasts from it. This paper constitutes a report on further yeast isolates from dough.

MEDIA AND METHODS

Most of the media and methods employed in this investigation were the same as reported by AHMAD, CHAUDHURY & AHMAD (1954). The additional media that were used were according to LODDER & KREGER-VAN RIJ (1952). For the study of sporulation of yeasts, standard presporulation medium, developed by AHMAD and his colleagues (1957), was used.

EXPERIMENTS AND RESULTS

Dough was obtained from three districts of East Pakistan namely Chittagong, Sylhet and Rangpur. Twenty strains were isolated from the dough obtained from Chittagong, thirty eight from Sylhet and thirty four from Rangpur. These strains were tested for their different morphological and physiological characters and identified.

Of the 92 isolates 88 were found to be yeasts and 4 to be members of fungi imperfecti. The 88 yeasts fell into four genera: *Saccharomyces*, *Candida*, *Torulopsis* and *Hansenula*. The four isolates belonging to fungi imperfecti fell into two genera, *Cladosporium* and *Sporophora*.

Genus *Saccharomyces*: All the 53 isolates belonging to this genus proved to be *S. carlsbergensis* HANSEN. Cells were round, short-oval and oval measuring (4—8) × (5—13) μ. After culturing for 3 days only a sediment was formed but after a month a ring also appeared.

TABLE I.

Fermentation capacities of 92 isolates.

+ Capacity to ferment within 48 hours present; — Capacity to ferment in 48 hours absent.

Designation of species	Designation of strains	No. of strains	Capacity to ferment								Total
			Glucose	Sucrose	Raffinose	Galactose	Maltose	Melibiose	Lactose	alpha-methyl glucoside	
Saccharomyces carlsbergensis	C_{35}I-IV, C_{36}I-V, C_{37}I-V, C_4-I-IV, C_{41}I-IV, C_{43}I-IV, C_{44}I-IV, C_{45}I-III, C_{46}I-IV, C_{48}I-III	40	+	+	+	+	+	+	—	—	
	C_{38}I-V, C_{39}I-IV, C_{42}I-IV	13	+	—	—	—	—		—	+	53
Candida krusei	C_{47}I-IV, C_{52}I-III, C_{53}I-IV, C_{54}I-IV	15	+	—	—	—	—		—	—	15
C. guilliermondii var. *membranaefaciens*	CI_{52}V, C_{56}I-IV, C_{57}I-III	8	+	+	+	+	—	—	—	—	8
Torulopsis colliculosa	C_{49}I-IV, C_{50}I-II	6	+	+	+	—	+		—	+	6
T. globosa	C_{50}III-IV	2	+	+	+	—	+		—		2
Hansenula anomala	C_{51}I-IV	4	+	+	+	+	+		—	—	4
Cladosporium butyri	C_{55}I-III	3	+	+	—	+	—		—	—	3
Sporophora sp.	C_{35}V	1	—	—	—	—	—		—	—	1

After one month at room temperature the streak culture was cream coloured, dull glistening, rather flat and almost smooth; and its margin was slightly lobated. No mycelium formation was found when slide culture test for mycelium formation was made. All the strains gave sporulation and formed one to four spored asci. All of them fermented glucose, sucrose, raffinose, galactose, maltose and melibiose but failed to ferment lactose (Table I) 13 strains (C_{38}I, C_{38}II, C_{38}III, C_{38}IV, C_{38}V, C_{39}I, C_{39}II, C_{39}III, C_{39}IV, C_{42}I, C_{42}II, C_{42}III, C_{42}IV) fermented α-methyl glucoside and the rest did not (Table I). All the 53 isolates assimilated glucose, sucrose, galactose, and maltose but lacked the capacity to assimilate lactose and potassium nitrate (Table II), and failed to utilise ethanol. Vitamin synthesis tests showed that out of the 53 isolates 47 were autotrophic, 5 had little growth in pantothenicless and one had little growth both in pantothenicless and thiaminless media (Table III).

Genus *Candida*: 23 isolates belonged to this genus. Of these 23 strains 15 were identified as *C. krusei* and 8 as *C. guilliermondii* var. *membranaefaciens*.

C. krusei (CAST.) BERKHOUT: All the 15 strains formed a thin, fragile somewhat powdery grey, slightly wrinkled pellicle in 2 % malt. The pellicle was found to be creeping up against the glass wall of the tube and a little sediment was also formed. Some difference was observed between bottom cells and cells forming the pellicle. The pellicle had only two types of cells, oval and elongated; amongst the bottom cells were found four types of cells namely round, oval, elongated and cylindrical. Secondly the pellicle cells were more or less uniform whereas the bottom cells presented all grades of intermediate types between the 4 types of cells met with. Measurement of pellicle cells was as follows: Oval cells measured (5.8—7.7) μ × (5.8—7.7) μ and elongated cells measured (2.9—4.4) × (6.7—9.6) μ. Measurement of bottom cells was as follows: elongated to cylindrical cells measured (1.9—3.8) × (7.7—11.8) μ, small cells measured (2.9—4.9) × (3.8—7.7) μ, and very long and thin cylindrical cells measured (1.9—2.9) × (7.7—32.8) μ. The streak culture was soft, flat, dull, smooth and yellowish. The margin of the streak culture was sinuous with pseudomycelial fringes. There was a rich development of pseudomycelium. The development of mycelium on peptone-glucose-agar was different from that on potato-agar. The blastospores on peptone-glucose-agar were elongated and cylindrical. They were developed in verticils composed of erect branched chains. On potato agar the mycelium was much branched with spherical verticils of blastospores. None of them gave sporulation. All of the 15 strains fermented glucose and lacked the capacity to ferment sucrose, raffinose, galactose, maltose, lactose and α-methyl glucoside (Table I). All the strains assimilated glucose but failed to assimilate sucrose, galactose, maltose, lactose and potassium nitrate (Table II). They formed a moderately thin, yellowish, dull pellicle and a sediment in a medium containing ethanol as the only source of carbon.

TABLE II.

Assimilation capacities of 92 isolates.

+ Capacity of assimilation present; — Capacity of assimilation absent.

Designation of species	No. of strains	Capacity to assimilate					
		Glucose	Sucrose	Galactose	Maltose	Lactose	Potassium nitrate
Saccharomyces carlsbergensis	53	+	+	+	+	—	—
Candida krusei	15	+	—	—	—	—	—
C. guilliermondii var. *membranaefaciens*	8	+	+	+	+	—	—
Torulopsis colliculosa	6	+	+	—	+	—	—
Torulopsis globosa	2	+	+	—	+	—	+
Hansenula anomala	4	+	+	+	+	—	+
Cladosporium butyri	3	+	+	+	+	+	+
Sporophora sp.	1	+	+	+	+	+	+

None of these strains were found to coagulate milk. They, however, made it alkaline. None of the strains formed any esters. They also did not split fat and produce starch. Vitamin synthesis tests showed that they were all autotrophes (Table III).

Candida guilliermondii (CAST.) LANGERON et GUERRA var. *membranaefaciens*: The 8 strains belonging to this species had three types of cells namely small round short-oval cells; round giant cells; and long pseudomycelial cells. The small round and short oval cells measured (1.9—3.8) × (8.7—10.5) μ. They formed a sediment and a dull pellicle in 2 % malt. After one month there was a thick pellicle in the malt extract. All of them formed shining cream coloured streak. Slide culture test showed rich development of pseudomycelium and blastospores in all of them and the development of pseudomycelium was of the "Mycocandida" type. They fermented glucose, sucrose, raffinose, galactose and did not ferment maltose, melibiose, lactose and α-methyl glucoside (Table I). They assimilated glucose, sucrose, galactose maltose and did not assimilate lactose and potassium nitrate (Table II). When ethanol was used as the sole source of carbon, they were found to grow well and to form a pellicle. Litmus milk with and without calcium lactate acquired a blue colour within a week of inoculation of these yeasts. They failed to split fat and produce starch. When tested for the synthesis of various members of vitamin B complex, they proved to be autotrophic (Table III).

Genus *Torulopsis*: 8 isolates belonged to this genus. Of the 8 isolates, six were identified as *T. colliculosa* and 2 as *T. globosa.*

Torulopsis colliculosa (HARTMANN) SACCARDO: All the six strains of this yeast had round to oval cells measuring (3.8—5.8) × (5.8—5.8) μ. They were found to multiply by means of budding. They formed only sediment but no pellicle in 2 % malt. These yeasts formed cream coloured streak which was almost smooth with a few warts. The margin of the streak culture was slightly sinuous. They did not form any mycelium. They fermented glucose, sucrose, raffinose, maltose and α-methyl glucoside but lacked the capacity to ferment galactose and lactose (Table I). They assimilated glucose, sucrose and maltose but failed to assimilate galactose, lactose and potassium nitrate (Table II). They failed to utilise ethanol as a sole source of carbon. They coagulated litmus milk after 36 hours of inoculation but afterwards the coagulated milk was gradually digested within three weeks. They failed to split fat and produce starch. When tested for the synthesis of various members of vitamin B-complex, all of them were found to be autotrophic (Table III).

Torulopsis globosa (OLSON et HAMMER) Nov. comb: The 2 strains of this yeast which were isolated, had round and oval cells measuring (4.0—5.5) × (5—7) μ. They formed a sediment and no pellicle in 2 % malt. These isolates formed cream coloured streaks. The margin of the streak culture was slightly sinuous. Slide culture test for mycelium formation showed that they did not form any mycelium. Both the isolates fermented glucose, sucrose, raffinose, maltose and lacked the capacity to ferment galactose and lactose (Table I). The two isolates assimilated glucose, sucrose, maltose, and potassium nitrate but lacked the capacity to assimilate galactose and lactose (Table II). There was little growth when ethanol was used as the sole source of carbon. When they were tested for the synthesis of various members of vitamin B-complex they were found to be autotrophic (Table III).

Genus *Hansenula*: 4 isolates belonged to this genus. All of them fell under a single species, *H. anomala.*

Hansenula anomala (HANSEN) H. et P. SYDOW: All the strains had round, short-oval, and oval cells measuring (2.9—4.9) × (3.8—5.8) μ. They formed a white, dull, thin, smooth fragile creeping pellicle and a sediment in 2 % malt. After one month a thick pellicle was formed. They formed white, soft, dull smooth streaks. The margin of the streak culture was lobate and mostly fringed with pseusdomycelium. There was little sporulation, 1—4 spores per ascus were found. All the 4 strains fermented glucose, sucrose, raffinose, galactose, and maltose but did not ferment lactose and α-methyl glucoside (Table I). They assimilated glucose, sucrose, galactose, maltose and potassium nitrate but failed to assimilate lactose (Table II). They were found to grow well in a medium containing ethanol as the sole source of carbon. The litmus milk was not coagulated by this yeast, but its colour changed to blue. These

TABLE III.

Vitamin requirements of 92 isolates

+ Synthesiser; — Nonsynthesiser.

Designation of species	Designation of strains	No. of strains	Growth in synthetic media									Total
			Basal solution	Pantothenic less	Biotin less	Pyridoxinless	Inositolless	Niacinless	Thiaminless	Riboflavinless	All Vitamins	
Saccharomyces carlsbergensis	C_{35}II-IV, C_{36}I-V, C_{37}I-IV, C_{38}I, C_{38}III-V, C_{39}I-IV, C_{40}II-IV, C_{41}I-IV, C_{42}I-IV, C_{43}I-III,											53
	C_{44}I-IV, C_{45}I-III, C_{46}I-IV, C_{48}I-II	47	+	+	+	+	+	+	+	+	+	53
	3_{37}V, C_{38}II, C_{40}I, C_{43}IV, C_{48}III	5	little	little	+	+	+	+	+	+	+	
	C_{35}I	1	little	little	+	+	+	+	little	+	+	
Candida krusei	C_{47}I-IV, C_{52}I-III, C_{53}I-IV, C_{54}I-IV	15	+	+	+	+	+	+	+	+	+	15
C. guilliermondii var. *membranaefaciens*	C_{52}IV, C_{56}I-IV, C_{57}I-III	8	+	+	+	+	+	+	+	+	+	8
Torulopsis colliculosa	C_{49}I-IV, C_{50}I-II	6	+	+	+	+	+	+	+	+	+	6
T. globosa	C_{50}III-IV	2	+	+	+	+	+	+	+	+	+	2
Hansenula anomala	C_{51}I-IV	4	+	+	+	+	+	+	+	+	+	4
Cladosporium butyri	C_{55}I-III	3	+	+	+	+	+	+	+	+	+	3
Sporophora sp.	C_{35}V	1	little	+	+	+	+	+	little	+	+	1

TABLE IV.

Isolation and distribution of 10 *yeasts and* 2 *members of fungi imperfecti from dough obtained from* 7 *districts of East Pakistan. The three new districts, isolates from which have been reported in this paper, have been marked with an asterisk.*

Designation of species	*Rangpur	Rajshahi	Pabna	Dacca	Comilla	*Chittagong	*Sylhet	Total
1. *Saccharomyces carlsbergensis*	—	2	7	18	15	19	34	95
2. *S. cerevisiae*	—	—	3	4	—	—	—	7
3. *S. cerevisiae* var. *ellipsoideus*	—	8	8	10	—	—	—	26
4. *Candida krusei*	11	—	—		—	—	4	15
5. *Candida guilliermondii*	—	—	—	2	—	—	—	2
6. *C. guilliermondii* var. *membranaefaciens*	8	—	—	—	—	—	—	8
7. *Torulopsis colliculosa*	6	—	—	—	—	—	—	6
8. *T. globosa*	2	—	—	—	—	—	—	2
9. *Hansenula anomala*	4	—	—	—	—	—	—	4
10. *Rhodotorula glutinis*	—	—	—	2	—	—	—	2
11. *Cladosporium butyri*	3	—	—	—	—	—	—	3
12. *Sporophora* sp.	—	—	—	—	—	1	—	1
Grand Total	34	10	18	36	15	20	38	171

yeasts failed to split fat and produce starch. All the strains were found to synthesise all the seven members of vitamin B-complex when they were tested (Table III).

Genus *Sporophora*: Only one isolate belonged to this genus. As it is known, this genus has been created by LUTERAAN (1952) [1]. Like the genus *Cephalosporium*, the genus *Sporophora* represents the conidial states of different perfect fungi. Only one species under this genus namely the type species *Sporophora toruliformis* LUTERAAN, has been described. The strain isolated by the authors resembles very much the species *toruliformis* but there are some differences in spore size and in the development of colonies. Hence this fungus has been identified for us by Dr. JOHANNA WESTERDIJK as *Sporophora* species.

Sporophora species: In malt extract solution a sediment was formed and the cells were cylindrical, slender and spindle-shaped. The streak culture was light brown, soft and wrinkled. Mycelial fringes were present. When slide culture test was made this fungus dit not ferment any of the sugars tested (Table I), but assimilated glucose, sucrose, galactose, maltose, lactose and potassium nitrate (Table II). It was found to grow well in a medium containing ethanol as the sole source of carbon. It split fat but did not produce starch. When tested for the various members of vitamin B-complex, it showed little growth in thiaminless and good growth in the rest (Table III).

Genus *Cladosporium*: 3 isolates belonged to this genus. All the three strains were identified as *Cladosporium butyri*.

Cladosporium butyri ORA JENSEN: They formed a mucous, partial pellicle and a sediment in 2 % malt solution. True mycelium was formed profusely by them. Single cells were oval, long-oval and cylindrical. After one month a thick black pellicle was formed. They fermented glucose, sucrose and galactose but did not ferment raffinose, maltose, lactose and α-methyl glucoside (Table I). They showed the capacity to assimilate glucose, sucrose, galactose, maltose, lactose and potassium nitrate (Table II). When ethanol was tested as the sole source of carbon, they were found to grow in it. They split fat but did not form starch. All of them synthesized the various members of vitamin B-complex (Table III).

DISCUSSION

Saccharomyces cerevisiae (HENRICI et al. 1947), *Candida krusei* (REDAELLI, CASTELLI & CIFERRI 1938) and *Saccharomyces chevalieri* (SCHAEFFER) have been claimed to be associated with the raising of dough. AHMAD & KHAN (1955) added to this list three more yeasts namely *Saccharomyces carlsbergensis*, *S. cerevisiae* var. *ellipsoideus*, and *Candida guilliermondii*. They further stated that while *Saccharomyces cerevisiae* is most commonly associated with the raising of dough in Western countries, in East Pakistan it was *S. carlsbergensis* which was the main baker's yeast.

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[1]) Personal communication from Dr. JOHANNA WESTERDIJK, Baarn, Holland.

Of the 92 organisms further isolated by the authors from dough obtained from three more districts of East Pakistan, 88 were yeasts and 4 fungi imperfecti. The yeast comprised 53 strains of *Saccharomyces carlsbergensis*, 15 strains of *Candida krusei*, 8 strains of *Candida guilliermondii* var. *membranaefaciens*, 6 strains of *Torulopsis colliculosa*, 2 strains of *Hansenula anomala*. The fungi imperfecti comprised 3 strains of *Cladosporium butyri* and one strain of *Sporophora* sp. (Table IV). Since *Sporophora* failed to ferment glucose, sucrose raffinose, galactose, maltose, and lactose, it is concluded that it is not responsible for the raising of the dough and is present as a contaminant.

The absence of *Saccharomyces cerevisiae* from and predominance of *S. carlsbergensis* strains amongst these isolates were note-worthy, and confirm the findings of AHMAD & KHAN (1955) that it is not *S. cerevisiae* but *S. carlsbergensis* which is mainly responsible for the raising of the dough in East Pakistan. It is further seen that a larger variety of organisms participate in the raising of the dough than was reported in 1955. Thus amongst the present isolates are not only found new yeasts, namely *Candida krusei*, *Candida guilliermondii* var. *membranaefaciens*, *Torulopsis colliculosa*, *Torulopsis globosa*, and *Hansenula anomala* but also one member of fungi imperfecti namely *Cladosporium butyri*. It may be mentioned that there has been no report of isolation of *Candida guilliermondii* var. *membranaefaciens*, *Torulopsis colliculosa*, *Torulopsis globosa*, *Hansenula anomala* and *Cladosporium butyri* from dough.

When the distribution of yeasts isolated previously (AHMAD & KHAN 1955) and now by the authors from dough, is studied (Table IV) it is seen that frequency of *S. carlsbergensis* increases as one goes from drier western districts to moister eastern districts.

Fermentation of α-methyl glucoside provides an additional character for both taxonomy and genetics of yeast. Its use in taxonomy may be limited since among the 53 strains of *S. carlsbergensis*, reported in this paper there were found to be both fermenters and nonfermenters. However, all the strains of *Candida krusei*, *Candida guilliermondii* var. *membranaefaciens*, *Hansenula anomala* and *Cladosporium butyri* were non-fermenters of α-methyl glucoside where as all the strains of *Torulopsis colliculosa* fermented α-methyl glucoside.

Tests for the synthesis of various members of vitamin B-complex showed that all the 92 isolates were more or less autotrophic.

SUMMARY

88 strains of yeast and 4 strains of fungi imperfecti have been isolated from dough from three more districts of East Pakistan. The yeasts comprise 53 strains of *Saccharomyces carlsbergensis*, 15 strains

of *Candida krusei*, 8 strains of *Candida guilliermondii* var. *membranaefaciens*, 6 strains of *Torulopsis colliculosa*, 2 strains of *Torulopsis globosa*, and 4 strains of *Hansenula anomala*. Of the four strains of fungi imperfecti, 3 belonged to *Cladosporium butyri* and one to *Sporophora* sp. The *Sporophora* sp. is considered to be a contaminant.

The 92 isolates have been tested for their capacity to ferment α-methyl glucoside. The possibility of the utilisation of the fermentation of α-methyl glucoside as an additional character in yeast taxonomy has been discussed.

Tests for the syntheses of various members of vitamin B-complex have shown that all the 92 isolates are more or less autotrophic.

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DEBARYOMYCES ARTAGAVEYTIAE N.SP. ASSINALADO EM SECREÇÃO GENITAL DE MULHER

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O gênero *Debaryomyces* Lodder & Van Rij, estabelecido, em 1909, por Klocker, manteve-se em estado de confusão, abrigando espécies de morfologia e conduta bioquímica muito dissemelhante, até à sua revisão e emenda, procedidas por Lodder & Van Rij, em 1952. Conservado o seu nome, apesar da exclusão da espécie tipo, *D. globosus* Klock., modificaram-se, no entanto, as suas características e o heterogêneo grupo de fungos que compreendia se reduziu a apenas 5 espécies.

Nesse gênero, descrevemos, agora, *Debaryomyces artagaveytiae* n. sp., tendo, como tipo, uma cêpa isolada de secreção genital feminina, de paciente vítima de vaginite.

O nome específico é dado em homenagem ao ilustre zimólogo uruguaio, Dr. Ricardo C. Artagaveytia-Allende, da Faculdade de Humanidades e Ciências, de Montevideo.

Essa nova espécie distingue-se pelos seguintes caracteres:

Cultivo em extrato de malte:

Aos 3 dias, ausência de anel, de película e de sedimento. Blastosporos globosos, de 3—5 μ de diam., ovoides ou ogivais, frequentemente com discretas saliências polares e, também, longo-ovais, de 2,5—5 × 4—7 μ. Brotação simples, catenulada e múltipla. Aos 20 dias, anel esboçado, irregular, blastosporos de paredes às vezes demasiadamente espessas e de duplo contôrno, globosos ou ovais, de 3—5 × 4—7,5 μ; as células de duplo contôrno apresentam uma deiscência, por onde escapa uma nova célula constituida („durable cells").

Em água de batata, com bile de boi: aos 3 dias, ausência de anel, película e sedimento, notando-se a mesma morfologia microscópica observada em extrato de malte, aos 3 dias. Aos 20 dias, raras células de paredes duplas e poucas „durable cells".

Colônias em spot-mat:

Sôbre Sabouraud-dextrose-agar: aos 15 dias e à temperatura ambiente (28—30° C), colônia-gigante branca ou levemente cinza, sub-circular, 10—12 mm de diam., plana, com discreta elevação central, lisa, mas com leves sulcos no sentido radial, os quais não atingem o centro; bordos ondulados largamente, bem definidos; ausência de filamentação. Reverso branco-amarelado, liso.

Sôbre Hansen-agar: aos 15 dias e à temperatura ambiente (28—30° C.), colônia gigante, sub-circular, 8—10 mm de diam., plana, lisa, cinza-claro, brilhante; bordos ondulados, bem definidos; ausência de filamentação; reverso amarelado, liso.

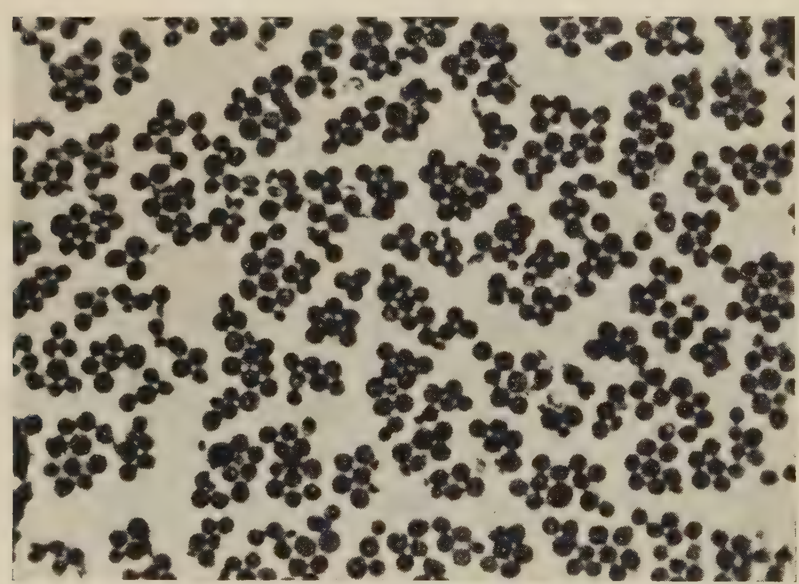

Fig. 1. *Debaryomyces artagaveytiae* Bat., J. Silveira & Coelho n. sp. Sôbre malte-agar.

Em batata agar: aos 15 dias e à temperatura ambiente (28—30° C.), colônia-gigante circular, de 10—12 mm., branco-acinzentada, sem brilho, plana, mas com leve intumescência central, lisa, bordos inteiros, sem filamentação; reverso branco, liso.

Sôbre Malte-agar: aos 15 dias e à temperatura ambiente (28—30° C.), colônia irregular, mas tendendo para a fórma circular, plana, com leve intumescência central, branco-cinza, de pouco brilho; bordos sulcados fracamente, lobados ou erosados; reverso amarelado, liso; ausência de filamentação. Blastosporos globosos, ovais ou longo ovais, 3—6×4—7 μ, Fig. 1.

Cultivo em:
Gelatina: aos 15 dias, não liquefazem a gelatina.
Amido agar: aos 15 dias, não desdobram o amido.
Etanol, como única fonte de carbono: — não crescem, observa-se ausência de anel, de película e sedimento.
Meio de nitrato: aos 15 dias, não reduzem o nitrato.
Leite de litmus: aos 6 dias, inalterado. Aos 15 dias, alcalinizam pouco o meio.

Cultura em lâmina de batata: aos 8 dias, pseudomicélio rudimentar, mas, relativamente, abundante, formado por células curto-ovoides, ovais, ogivais e estalagmoides. Não há diferenças entre as células pseudo-miceliais e os blastosporos.

MICROMORFOLOGIA SOBRE MEIO SÓLIDO, À TEMPERATURA AMBIENTE (29—30° C.), A 3 DIAS

Sabouraud dextrose-agar: células globosas, em sua maioria, e células curto-ovais e ogivais, de 2—5 × 3—5,5 μ. Células longo-ovais com 2,5—3,5 × 5—7,5 μ. Brotação simples, múltipla e catenulada. Células com paredes de duplo contôrno, globosas, e células globosas ou ovais, apresentando uma deiscência, por onde escapa nova célula constituida. Estes dois tipos têm as dimensões de 5,3—4 × 5—7,5 μ.

PROVAS BIOQUIMICAS

Zimograma			Auxonograma			
Glucose	—	—	Glucose	+	Sulfato de amônio	Nitrato de potássio
Galactose	—	—	Galactose	+	+48h	—48h
Sacarose	—	—	Sacarose	+		
Maltose	—	—	Maltose	—		
Lactose	—	—	Lactose	+		
	10d	20d		48h		

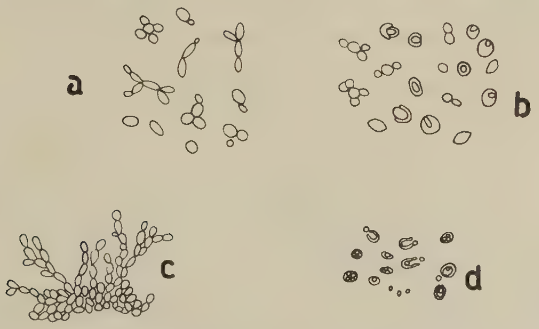

Fig. 2. *Debaryomyces artagaveytiae* BAT., J. SILVEIRA & COELHO n. sp.
a) em extrato de malte; b) sôbre sabouraud-dextrose-agar; c) sôbre lâmina de batata; d) ascos e ascosporos.

Não fermenta, portanto, os açúcares: Glucose, galactose, sacarose, maltose e lactose.

Assimila a glucose, galactose, sacarose e lactose; não assimila a maltose, nem o nitrato de potássio.

Esporulação

Aos 15 dias, raros ascos curto-ovais ou ovoides, de 3—4 × 4—6 μ, formados sob conjugação heterogênica, 1—4-espórico, Fig. 2.

Ascosporos globosos curto-ovais, de membrana lisa, aparentemente, e de 1 μ de diam.

O fungo em tela foi isolado de secreção genital feminina, de M.R.S., tipo, 2201, em cultura e sôbre papel, no I.M.U.R. Leg. J. S. SILVEIRA, 25/3/959.

DEBARYOMYCES ARTAGAVEYTIAE N. SP.

In musto maltato cellulae globosae, 3—5 μ diam., vel ovoideae, ogivales et longo-ovoideae, 2,5—5 × 4—7 μ, brotationem simplicem, catenulatam vel multiplam ostendens; annulus, pellicula et sedimentum non sunt. In agaro-maltato cellulae globosae, ovoideae vel longo-ovoideae, 3—6 × 4—7 μ, sine mycelio vel pseudomycelio. Fermentatio nulla; glucosis, galactosis, saccharosis, lactosis assimilantur; maltosis et nitras kalicus non assimilantur. Asci ovoidei, 1—4-spori, 3—4 × 4—6 μ. Ascosporae globosae vel ovoideae, levigatae, 1 μ diam. Typus, 2201, in culto et in chartis, I.M.U.R. In secretione genitali fem.

A cepa de *Debaryomyces artagaveytiae* n. sp., ora em estudo, foi assinalada sôbre secreção vaginal de M.R.S., brasileira, casada, residente no Recife, que tinha as seguintes queixas: corrimento vaginal, dôres abdominais; tivera o último mênstruo havia quatro mêses e fizera uso de penicilina e dihidro-estreptomicina; achava-se, também, grávida, há 5 mêses.

Ao espéculo, vagina e colo do útero banhados por abundante fluxo amarelo, viscoso; colo do útero erosado.

As pesquisas laboratoriais proporcionam os seguintes valores: ph-7; ausência de *Trichomonas*, flora vaginal do tipo IV, presentes células leveduriformes; cultivo positivo, originando *D. artagaveytiae* n. sp.

A paciente foi submetida a tratamento, com o emprêgo de Colpomicina, em comprimidos vaginais. Após 16 dias de uso da medicação, foi submetida a novo exame, revelandose, então, fluxo escasso, ph-5, *Trichomonas* negativo, flora vaginal do tipo III, com ausência de células leveduriformes; semeadura, nos meios de laboratório, negativa, havendo, assim, perfeito contrôle do fungo.

A análise bibliográfica que efetuamos, em busca de informações sôbre a patogenicidade de espécies conhecidas de *Debaryomyces*, demonstrou-nos que êsse gênero, segundo o conceito restrito de LODDER & VAN RIJ, tem expressivo destaque na Micopatologia humana.

Assim, LODDER & VAN RIJ, apoiados em vários autores, referem: *D. hansenii* (ZOFF.) LODDER & VAN RIJ está relacionado com um caso de blastomicose do tipo Gilchristia (Farina, 1929).

D. kloeckeri GUILLIERMOND & PEJU, 1919, tem estado associado a um caso de angina (Peju);

idem, de blastomicose de foco múltiplo (FONSECA, 1922);
idem, de infecção da pele (OTA, 1923);
idem, de carcinoma de ovário (OTA, 1923);
idem, de 2 casos de micose interdigital (OTA, 1924);
idem, de infecção de unhas (OTA, 1924);
idem, de queratite (OTA, 1923);
idem, de fezes de um paciente com helmintíase (GRIGORAKI & PEJU, 1921);
idem, de micose interdigital (OTA, 1924);
idem, de líquido obtido por punção lombar (BACHINSKY, 1929);
idem, de abcesso pulmonar de um paciente morto de tuberculose (KUONKOTINA & KRASSIKUKOW);
D. subglobosus (ZACH) LODDER et VAN RIJ, 1934, isolado: de doença de unha (ZACH, 1935);
idem, da garganta de um paciente, que tinha sofrido de tuberculose (ORIE, 1941);
idem, de esputo (HOLANDA, 1947).

SUMMARY

This paper deals with the yeast *Debaryomyces artagaveytiae* BAT., J. SILVEIRA & COELHO n. sp. found in woman genital secretion.

This fungus is characterised by its biochemical ability to grow in sugar media having glucose, galactose, saccharose and maltose but it does not fermentate these sugars, nor maltose.

The mycelium is lacking but through an isogamic conjugation asci are developed, containing from 1 to 4 globose ascospores.

The infected woman has been treated successfully with colpomicin.

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STUDIES ON THE SPORULATION OF PHYTOPHTHORA PARASITICA DASTUR VAR. PIPERINA DAST. RESPONSIBLE FOR LEAF AND FOOT-ROT OF PAN, PIPER BETLE. L.

by

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(Jute Research Institute, Tejgaon, Dacca, East Pakistan)

(with 1 fig.)

(20.IX.1960)

Introduction

In East Pakistan pan is one of the important crops and a large section of people earn their living on pan cultivation. This plantation crop is cultivated in shades within enclosures made of bamboo and other thatches known as Baroj. The cool and humid condition of a Baroj favour a big list of pathogens, some of them at times becoming very much virulent on pan. There are fungi which cause damage all the year round, others, however, at certain season appear in epiphytotic condition resulting complete destruction of vines of a Baroj.

Phytophthora parasitica var. *piperina* is a fungus of the latter group and by far the most important one causing disease at the close of a rainy season and during autumn. The disease appears with first noticeable symptom as water soaked lesions on leaves while the leaf is in green stage. One to two days later the lesion increases with characteristic concentric rings on the affected leaf. Subsequently, leaves nearby also get infected and damaged. On the stem water soaked marks appear which in two days turn brown causing lesion. Consequently, the affected vines droop and dry up.

The pathogen produces characteristic swarm-spores within sporangia on the lesions in presence of free water. Temperature and humidity seem to be responsible for this production of zoospores and initiation of infection by the germinated spores of this organism as has been observed with different fungi by many workers (1), (2), (3), (4).

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[1]) Now at Texas A & M College, U.S.A.

EXPERIMENTS AND RESULTS

Vegetative mycelial felt of *Phytophthora parasitica* obtained from a 7-day old culture on pea meal formed the mycelial stock of the organism. From such mycelial stock a number of small mycelial bits whenever necessary were taken and exposed to different temperature and humid conditions for the production of sporangia, zoospores and their germination.

Effect of temperature on sporangial production

From cleaned (by washing with sterilized water) mycelial mat a number of equal pieces was made for incubating them for 72 hours at different temperatures. For each temperature replicate petridishes having 20 ml sterilized water in each with a few pieces of mycelial bits were used. After 72 hrs. random samples were drawn from each replication, mounted on cotton blue glycerine and five microscopic fields from each sample were studied (Table I).

TABLE I.

Effect of temperature on the production of sporangia.

Temp. in 0C.	No. of Microscopic fields (1.77 sq.mm)	Average No. of sporangia per field	Average No. of chlamydospores per field
5	15	0	0
10	15	0	18
15	15	0	25
20	15	12	36
25	15	20	10.77
26	15	26	6.5
27	15	32	0
28	15	31	0
29	15	30	2.52
30	15	30	3.33
31	15	16	0
32	15	0	0
35	15	0	0

The sets kept at 27, 28, 29 and 30° C produced more or less equally good number of sporangia than the sets kept at 25, 26 and 31° C. But no sporangia developed at 32° C.

Temperature and liberation of zoospores

Duplicate petridishes having arrangement for hanging drop culture for each of the different temperatures used here were kept in the respective incubators one hour before transferring the bits of sporangial culture (developed at a constant temperature of 29° C) on the water drop.

The hanging drop culture from each set was quickly taken out at regular intervals of ½ hr. exposure from the petridishes and examined under microscope to see if there was any empty sporangia. The results obtained during 3 hrs. observation were noted in Table II.

TABLE II.

Effect of temperature and time taken for the liberation of zoospores.

Temp. in 0C.	No. of microscopic field observed in each replication	Percentage of empty sporangia					
		30 min.	60 min.	90 min.	120 min.	150 min.	180 min.
17	15	0	0	0	0	0	0
19	15	0	5	10.5	25	0	0
21	15	5	20	35	45	0	0
23	15	5	20	34.5	44	0	0
25	15	0	5	7	12	0	0
27	15	0	0	0	0	0	0
28	15	0	0	0	0	0	0
29	15	0	0	0	0	0	0
31	15	0	0	0	0	0	0

Among the temperatures used 21 and 23° C appeared most suitable for liberation of zoospores. In both the cases time taken for liberation was at about 30 minutes when about 5 % sporangia were found empty. The number of empty sporangia gradually increased to 45 % during 120 minutes time. Innumerable motile zoospores were also found under microscope. Percentage of empty sporangia was found quite low with sets kept at 19 and 25° C. Time taken for liberation was also found beyond 30 minutes. No liberation was observed at temperature from 27 to 31° C.

Effect of humidity on sporangial formation

So far the development of sporangia was observed in free water. In this experiment it was intended to see the effect of atmospheric humidity on the production of sporangia.

Cleaned mycelial bits were gently placed over a semisoaked filter paper for draining superficial moisture without allowing the mat to dry up completely. Bits of mycelia 3 mm × 3 mm were cut from this mat and were thus ready for transfer to humidity chambers.

Well fitted petridishes having double filter paper were used as humid chambers and 10 ml redistilled water, sucrose solution and saturated salt solutions (1) supposed to set up respective percentage of relative humidities were added to respective petridishes. In each humid chamber a single perfectly dry slide had been kept over two glass rods so that direct touch with soaked filter paper did not happen. This was done one hour before the transfer of mycelial bits. The humid chambers were kept for 72 hrs. at a temperature of 28° C. The results obtained were tabulated in Table III.

Only at 100 % humidity where condensation of water was noticed there was sporangial formation. The frequency of bearing however,

TABLE III.

Effect of humidity on sporangial formation.

Percentage of relative humidity	Sample examined under microscope.	No. of microscopic field examined per sample.	Average No. of sporangia per microscopic field (1.77 sq. mm.)	Average No. of chlamydospores per microscopic field.
100	3	5	2	25
99.04	3	5	0	12
98.03	3	5	0	9
96	3	5	0	0

was very low. At other humidities where no such condensation prevailed, excepting chlamydospore-formation there was no sporangial development.

Effect of temperature on germination of zoospores

A number of hanging drop cultures with mycelial mats bearing sporangia were incubated at 21° C for 45 minutes to allow a certain percentage of zoospores to liberate. Immediately after 45 minutes replicate hanging drop cultures were exposed to different temperatures such as 18, 20, 22, 24 and 26° C.

The hanging drop cultures from each temperature were examined once after 60 minutes exposure and another after 90 minutes. The frequency of germinated spores as well as the length of germ tubes were measured and the average of 5 counts of the germinated spores within microscopic field and 10 measurements of germ tubes were noted in Table IV.

TABLE IV.

Effect of temperature on germination of zoospores.

Temperature	No. of spores counted after		No. germinated after		Percentage of germination after		Length of germ tube	
	60 m.	90 m.	60 m.	90 m.	60 m.	90 m.	60 m.	90 m.
18°C	120	130	0	0	0	0	0	0
20°C	112	128	0	100	0	78.1	0	18.04
22°C	132	124	108	112	81.81	90.3	17.26	37.44
24°C	127	127	0	101	0	71.65	0	17.26
26°C	130	120	0	0	0	0	0	0

From the results obtained the favourable range of temperature for germination of zoospores with the organism was found to be very narrow.

Effect of atmospheric humidity and free water on the liberation of zoospores

As above mycelial felt with sporangial bearings were chopped evenly into pieces (3 mm × 3 mm) and free water was soaked out with the help of sterilised half soaked filter papers. The mycelial bits thus prepared were transferred one each to rectangular cover glasses which were suspended 1 hour before inside the respective humidity chambers (1). In one additional set of chamber with 100 % relative humidity, mycelial felt with free water was suspended. All the sets were kept at 21-22° C in an incubator for 24 hours duration. The chambers were shaken gently at regular intervals taking care that no splashing of the solution within them occured, to minimise stratification of atmosphere or the solution within the chambers. At the end of the incubation mycelial felts on cover glasses were stained with cotton blue glycerine, mounted on slides and observed under microscope for making counts of the empty sporangia. Five counts were taken from five microscopic fields from one end of a cover glass to the other by pushing the slide sideways. The results obtained were tabulated in Table V.

TABLE V.

Effect of atmospheric humidity on liberation of zoospores.

Percentage of relative humidity	Condition of sporangia over cover glass	Number of sporangia counted.	
		empty	full
100	in free water	190	18
100	dry	23	179
99.24	dry	0	210
98.65	dry	0	206
98.03	dry	0	213

With all care and caution condensation of moisture could not be totally avoided in the chamber with 100 % humidity. When the cover glasses were taken out of this humidity chamber, the surface of the cover glass was found with condensation. The number of sporangia emptied in this case was 23 against 179 non-liberated sporangia. In similar condition of 100 % relative humidity when mycelial felt was kept in free water the number of empty sporangia were found in increased number, being 190 as against 18 non-liberated ones. No liberation of zoospores was observed with any other relative humidity tested here.

DISCUSSION

Temperature and humidity were found to have great influence on the pathogen, *P. parasitica* in the formation of sporangia, liberation and germination of zoospores. Sporangial formation was restricted within temperatures of 20—31° C (Table I). Atmospheric humidity of 100 % did not favour very much in developing sporangia (Table III). Free water appeared to be essential in sporangial formation. The ranges of temperature within which the liberation of zoospores took place were even narrower (Table II) than those required for sporangial development. Liberation of zoospores was found optimum both at 21 and 23° C though the range of temperature for their liberation was between 19 and 25° C. Similarly as with sporangial developmert atmospheric humidity as high as 100 % had very little effect on the liberation of zoospores unless there was condensation of water. Free water here was found a necessity in the liberation of zoospores (Table V), which is quite evident as the spores are motile and ciliated. The liberated zoospores in free water were found to germinate only within 20-24° C (Table IV) which was even more narrower than the range found favourable for liberation of zoospores (Fig. 1) At about 22° C the germination rate was found optimum. This low temperature requirement for germination shows why during summer (Av. temp. 30° C) when free water is so much available, disease fails to spread.

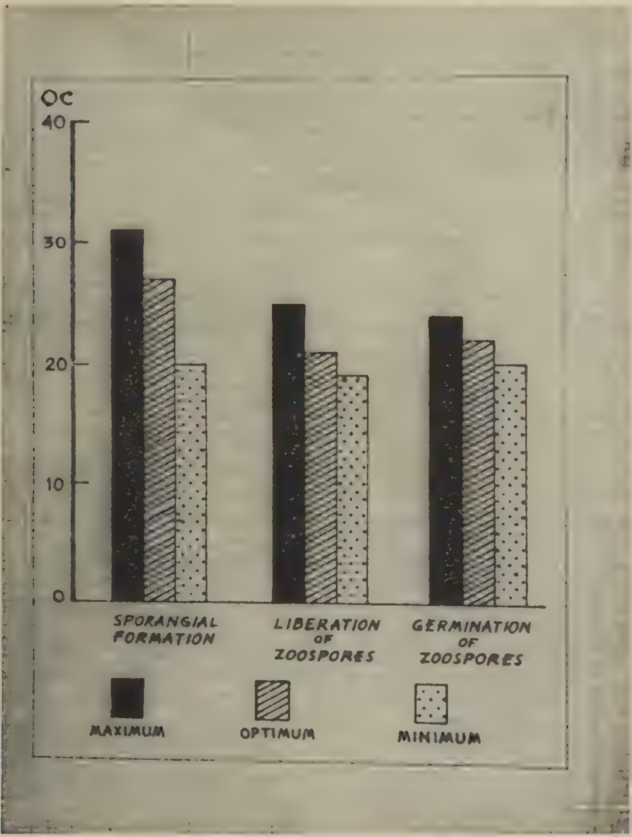

Fig. 1. Range of temperature for sporangial formation, liberation and germination of zoospores.

The overall result further appears to indicate why the disease spread all on a sudden. This is because development of sporangia continues throughout the wider range of temperature which remain as they are unless there happens a sudden fall of temperature when myriads of zoospores pushing out of the accumulated sporangia germinate and infect the host tissues as they come in contact developing characteristic lesions.

SUMMARY

Sporangial formation was found restricted within the range of temperature from 20-31° C. Atmospheric humidity of 100 % did not favour very much in developing sporangia. Free water appeared to be essential in sporangial formation. The range of temperature within which the liberation of zoospores took place was even narrower, 19 to 25° C. Temperature from 21 to 23° C appeared to be best condition for maximum liberation of zoospores. Similarly as with sporangial development atmospheric humidity as high as 100 % had very little effect on the liberation of zoospores unless there was condensation of water. The liberated zoospores in free water were found to germinate only within 20-24° C. At about 22° C the germination rate was found optimum.

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PIEDRA EN ECUADOR

por

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(con 7 figs.)

(30.VI.1960)

En la revisión que actualmente se realiza en esta Sección sobre Micosis superficiales, se hicieron algunas observaciones referentes a Piedra que ahora damos a conocer. El material estudiado fué de procedencia nacional, pero al hacer la relación se mencionan datos tomados de la bibliografía disponible.

Con alguna frecuencia se han encontrado antes en individuos de la ciudad y de los campos, nódulos piédricos en los cabellos, limitandose a la simple constatación, sin que se hubiera efectuado su estudio micológico, de modo que solo ahora podemos establecer particularidades desconocidas antes aquí.

Habiendose demostrado la existencia de las dos variedades, negra y blanca, se tratará de ellas separadamente.

PIEDRA NEGRA

Es interesante recordar que esta forma considerada primero como exclusivamente americana, fué posteriormente señalada en otros Continentes, en paises como Indonesia, Indochina, Malaya, Thailandia y en fin, fuera del hombre, en pieles curtidas de primates procedentes de Asia, Africa y América, existentes en las colecciones del Museo de Historia Natural de New-York, en la proporción de 44,5 % (Kaplan, 1959) (1).

En el Ecuador ésta es la forma mas comunmente encontrada en el Litoral, región situada en plena zona tropical, con un régimen de lluvias que pueden alcanzar una precipitación de un metro por año y con una elevada temperatura atmosférica; se halla pues entre las zonas con el llamado clima „asteriano" y en efecto, en estos sitios es frecuente la piedra negra sobre todo entre mujeres y niños, los que reconocen su presencia con distintos nombres como „pelotilla", „piedra de agua", liendre de agua", etc.

El nódulo. — Asienta en los pelos de la cabeza pero solo en su parte libre y en ella, debajo de la epidermícula, de modo que el desarrollo del hongo no compromete el crecimiento del pelo ni ocasiona su caida. Aparece en forma de nódulos de color negro (fig. 1), uno o varios en un cabello, de consistencia dura y de forma variable; cuando estan completamente desarrollados son cónicos con el extremo distal mas agudo, o son fusiformes, midiendo 1 mm mas o menos de largo y contorneando regularmente al pelo; cuando estan tiernos rodean parcialmente al cabello o aparecen como manchas diminutas en su superficie. Al tacto parece tratarse de granos de arena dificiles o imposible de desplazar.

El agente. — Por el aspecto del hongo en los nódulos, por la apariencia de sus colonias y por su micromorfología, consideramos que el agente es precisamente *Piedraia hortai* (BRUMPT) FONSECA & LEAO, 1928.

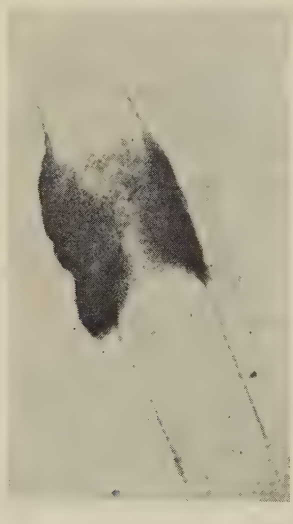

Fig. 1. Nódulo de Piedra negra.

Ciertamente que se han descrito por lo menos seis especies del género *Piedraia*, pero en opinión de CIFERRI, CHAVES BATISTA & SYLVIO CAMPOS (1956) (2), la única especie válida es *P. hortai*. Acaso el criterio unicista debe limitarse en atención al número de esporos dentro de un asco, pues parece que *P. venezuelensis* BRUMPT & LANGERON, 1934 con cuatro ascosporos por asco, ofrece un carácter botánico suficiente para mantenerlo como especie.

De la observación de los ascosporos cuyas fotografías se presentan luego, encontramos que por su forma, por la presencia de exosporio libre y por la apariencia de sus apéndices terminales, guardan mucha semejanza con los ascosporos de *P. javanica* BOEDIJN & VERBUNT, 1938 (3), aunque sus dimensiones se acercan a las señaladas por estos autores para *P. hortai* en material de Recife.

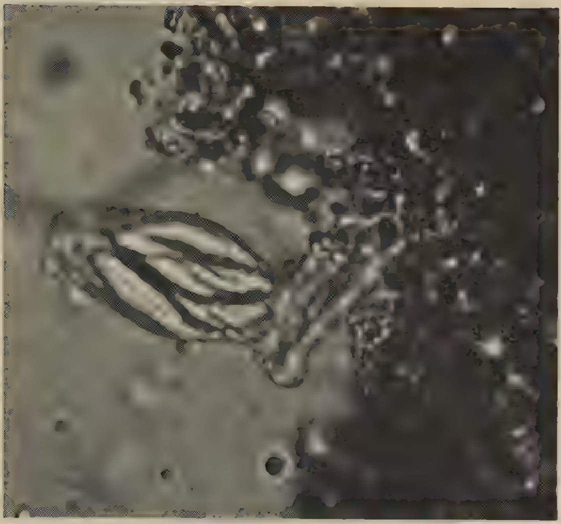

Fig. 2. Asco entero de *P. hortai* en pelo.

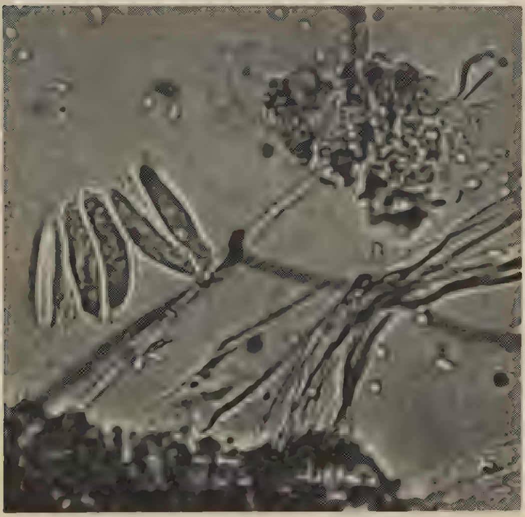

Fig. 3. Ascosporos libres de *P. hortai* (pelo).

En preparaciones húmedas en potasa al 10 %, hemos visto que los nódulos estan constituidos por un estroma compacto (ascostroma) de hifas oscuras y ramificadas dicotomicamente de 4 a 8 micras de diámetro unidas por una especie de cemento amorfo. Esto puede verse especialmente en los bordes de los nódulos jóvenes intactos, así como tambien su segmentación en artrosporos.

Cuando se disgrega un nódulo que esté bien desarrollado, pero nó cuando se deshace uno jóven, se encuentra en el interior del ascostroma, numerosos cuerpos ovales y claros que son los ascos los que contienen en su interior ocho elementos fusiformes o en banana, algo incurvados, que son los ascosporos y los cuales tienen un filamento o apéndice en cada extremo.

En las ilustraciones (figs. 2 y 3) se presenta un asco intacto y ascosporos aislados. Las dimensiones del asco fueron 43,12 por 26,62 micras y las de los ascosporos variaron entre 37,5 y 41,25 micras de largo, sin los apéndices, por 7,5 a 10,12 micras de ancho. En los extremos de los ascosporos se pudo apreciar mejor que su contenido estaba separado del exosporio y que los apéndices eran cortos y relativamente gruesos (fig. 4).

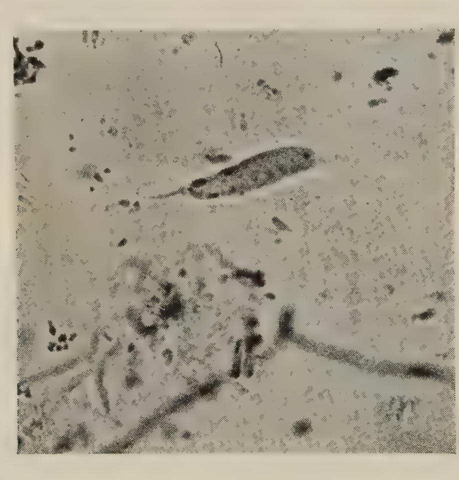

Fig. 4. Ascosporo aislado de *P. hortai*.

Cultivo. — Cuando se siembran nódulos en agar de Sabouraud a temperatura ambiente, aparecen colonias negras típicas que hemos obtenido, pero con cierta frecuencia a partir del mismo material desarrollan otras, vermiculares y amarillentas tipo *Trichosporon* o tipo JUHEL-RENOY cuya presencia ha sido interpretada de modo diverso. Para BRUMPT & LANGERON, se trataría de una asociación o infección mixta, posible porque en escamas de pitiriasis se encuentra comunmente *Trichosporon giganteum*, P. P. HORTA & NEGRONI (4) creen que las colonias negras provienen del desarrollo de los ascosporos y las de tipo *Trichosporon* de la parte vegetativa. En fin, el mismo NEGRONI sugiere la posibilidad de que ciertos *Trichosporon* serian las formas imperfectas de *Piedraia*.

En la figura 5 se ilustran esos dos tipos resultantes de dos nódulos que desarrollaron, en tanto que un tercero en el centro no prosperó. Los tres nódulos procedian de una misma persona y antes de sembrarlos fueron tratados por la penicilina.

La colonia negra es pequeña y dura, de superficie vellosa como el fieltro, con ligera elevación central y un halo velloso y laxo de color rojizo en la periferia; el pigmento se difunde en el medio de cultivo. Microscópicamente se ven hifas fuliginosas e hifas incoloras, las primeras gruesas de 4,7 micras, de las cuales salen brotes laterales cortos en ángulo recto o arqueadas, aparte de que en su trayecto aparecen clamidosporos y artrosporos. Las hialinas son delgadas de 1,72 micras y en muchas, sus lados son ondulados. En ocasiones pero no siempre estas colonias presentan ascos como en la vida parasitaria.

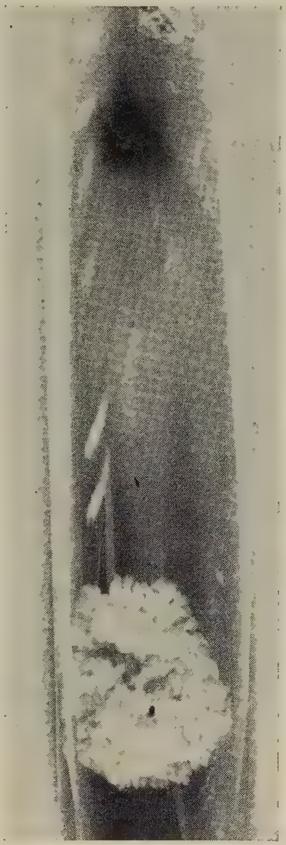

Fig. 5. Los dos tipos de colonias, en Piedra negra.

El otro tipo, o de JUHEL-RENOY, es blanco, de desarrollo extensivo, su superficie es cerebriforme y su textura blanda, es decir se asemeja a una colonia de *Trichosporon* como se describe en Piedra blanca, así como tambien en su micromorfología.

PIEDRA BLANCA

En Sud-América fué primero señalada en Colombia, despues en Brazil, Argentina, Venezuela, Paraguay y Uruguay y ahora en este país. En aquellas naciones así como aquí, su incidencia es menor que la de la Piedra negra.

El nódulo. — Es oval mas pequeño que el de la variedad anterior, por lo cual puede pasar desapercibido en un examen somero. No es blanco como pudiera creerse por su denominación, sinó de color pardo-rojizo, translúcido, de consistencia menos dura que el anterior y mas facilmente separable del cabello. Además, con frecuencia se acompaña de anomalías de los pelos parasitados como tricorrhexis o tricoptilosis.

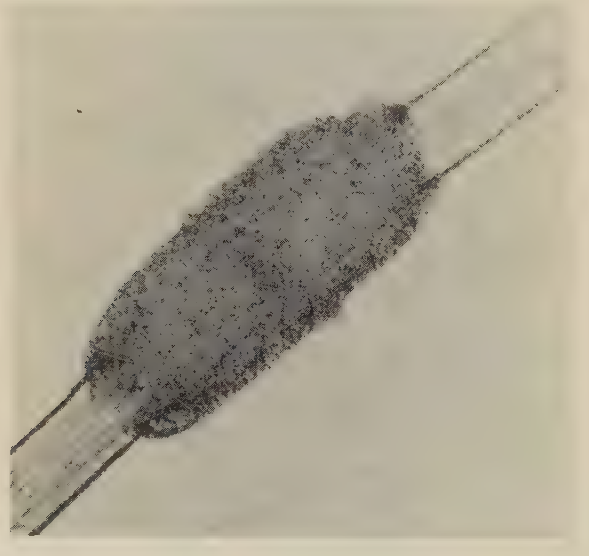

Fig. 6. Nódulo de Piedra blanca.

El hongo. — Es un fungi-imperfecti, *Trichosporon giganteum* Behrend, 1890, que asienta tambien debajo de la epidermícula del pelo, aunque Niño (1938) ha podido observar invasión intrapilar.

Al examen en potasa de un pelo parasitado se observa que el desarrollo fúngico puede rodearlo total o parcialmente y ese desarrollo es blanco mate (fig. 6). En su parte central se ven hifas hialinas que se componen de gruesos artrosporos rectangulares u ovoideos y tambien blastosporos de hasta 10 micras de diámetro, pero en ningún case se encuentran ascos; en la periferia de los nódulos se aprecia con frecuencia, como lo comprobamos, masas de cocos gram-positivos considerados como de infección secundaria y que quizá por su gran constancia significan verdaderos simbiontes del *Trichosporon*.

Cultivo. — El cultivo en Sabouraud glucosado es precóz, de color blanco al principio y de consistencia blanda. En el centro de la colonia bien desarrollada aparecen elevaciones vermiculares compactas y amarillentas y en los bordes un halo plano de color blanco sucio formado por hifas adherentes (fig. 7).

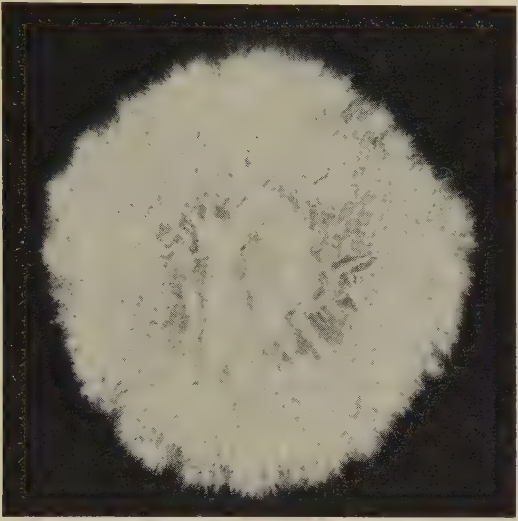

Fig. 7. Colonia de *T. giganteum* en Sabouraud glucosado.

Al examen microscópico del crecimiento en lactofenol azul, hemos visto hifas de 3,9 a 4 micras de grueso, numerosos artrosporos de 7,5 micras de largo por 4,87 micras de ancho, por término medio y abundantes blastosporos redondos de 5 micras de diámetro. Con frecuencia se ven blastosporos en gemación o emitiendo un tubo germinativo y en fin apresorios característicos en la parte central o en la periferia.

SUMARIO

Este trabajo se refiere a observaciones de Piedra en la República del Ecuador y es una parte de una revisión general sobre micosis superficiales que ahora se realiza en Guayaquil.

El estudio comprende las dos variedades, negra y blanca y además de la descripción de los nódulos, con sus particularidades microscópicas se describen tambien los cultivos obtenidos, en sus aspectos macroscópico y micromorfológico.

En Piedra negra se presentan detalles de los ascos y ascosporos de *P. hortai* (Brumpt) Fonseca & Leao, 1928. El A. encuentra cierta semejanza entre los ascosporos encontrados y los de *P. javanica* Boedijn & Verbunt, 1938. Se obtuvieron colonias negras típicas y además otras tipo *Trichosporon*, acerca de las que se recuerdan algunas interpretaciones.

En Piedra blanca se menciona su rareza. El organismo aislado se lo identifica con *T. giganteum* BEHREND, 1890 y se describen sus caracteres culturales y microscópicos.

SUMMARY

This work deals with some observations on Piedra in the Republic of Ecuador and it is a part of a general review about superficial mycoses which is now carried out in Guayaquil.

This study comprises the two varieties, black and white. Beside the description of the nodules, with its microscopic characteristics, the cultures are also described in both aspects: macroscopic and micromorphologic.

In black Piedra details of the asci are presented along with ascospores of *P. hortai* (BRUMPT) FONSECA & LEAO, 1928. The A. found certain resemblance between the ascospores described and those of *P. javanica* BOEDIJN & VERBUNT, 1938. Typical black colonies were obtained beside other *Trichosporon* type about which some interpretations are made.

In white Piedra its rarity is mentioned. The isolated organism is identified with *T. giganteum* BEHREND, 1890 and its microscopic and cultural characteristics are described.

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SEROLOGICAL STUDIES ON ASPERGILLUS FUMIGATUS

by

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INTRODUCTION

At present, serological studies on pathogenic fungi appear to be at a turning point toward a promising future. Species-specific antigen-antibody reactions are now introduced into the taxonomy of fungi, which hitherto had been based only on morphological findings. Also, the role of serological reactions in the diagnosis of mycoses has come to be reevaluated.

Until recently, no one attempted to study the serology of fungi either for taxonomic identification or for practical diagnosis, because of the great difficulty in obtaining a high titer immune serum and of too poor specificity. Although considerable improvements have been achieved in the serology of the species *Candida* chiefly by Japanese mycologists, in the case of *Asp. fumigatus* the difficulties have not yet been overcome. With sera of aspergillosis patients, Nicaud (1929) failed to demonstrate agglutination of the conidia. Using rabbit immune sera, Matsumoto (1929) obtained positive precipitation with culture filtrate antigen, but negative data with extracts of fungous cells. Using sera of rabbits immunized with cell sap of *Asp. fumigatus* and of *Cephalosporium acremonium*, Henrici failed to show precipitation of homologous cell saps. However, he obtained positive results by precipitin tests with *Asp. fumigatus* cell sap and the sera of the rabbits infected with its conidia. Matsumoto also observed positive complement fixation with culture filtrate antigen and the rabbit immune sera, though he could not clearly demonstrate its correlation to the taxonomy based on morphology. Aspergillin, devised by the National Institutes of Health (U.S.A.) for skin test antigen, has not yet been widely accepted because of its irritative effect and its poor specificity.

Since one of the present authors (M.F.) isolated a strain of *Asp. fumigatus* from a lung cavity of a penguin in 1953, the biological properties of the isolated mold from the standpoint of medical microbiology have been studied. It should be mentioned that by disintegrating cell walls through repeated cryolysis, the authors were able to obtain from the *Asp. fumigatus* pellicle a substance which was highly toxic to mice. Applying the same procedure to the formalin-killed mycelia of *Asp. fumigatus* and using the extract so obtained as an immunizing antigen the authors were able to obtain high-titer and highly specific rabbit immune sera. With these immune sera the specificity of various antigens prepared from the mold have been studied, the details of which are given in the following chapters.

I. PREPARATION OF IMMUNE SERUM

In our experiments *Asp. fumigatus* HANSHIN strain (isolated by one of the authors), No. 4040 strain[1]) and No. 4140 strain[1]) were used.

Three loopfuls of the conidia were inoculated into 130 ml of Czapek's fluid medium and cultivated at 37° C for 3 days, shaking the culture continuously. By this method, the mold does not develop conidia and grows to form a mass of mycelia. Formalin was added to 3 % and the culture was left at room temperature for another 3 days. After confirming the death of the mold, the mycelia were collected and ground with a mortar and pestle until pasty in appearance. The paste was then dried in chilled acetone, 0.5 mg of the acetone dried powder suspended in 5 ml of physiological saline frozen by immersing the tube into chilled acetone and then thawed in a water bath at 37° C. After this procedure was repeated 15 times, the supernate was separated by centrifugation. This extract will hereafter be called ME (an abbreviation for mycelial extract). 0.5 ml of ME (started from 50 mg of dried mycelia) could be injected into mice without giving any toxic effect.

Two rabbits were intravenously immunized with ME and its residue to a concentration of 0.5 mg per ml. The doses given were 0.5 ml for the first injection and 1 ml for the second to the tenth injection. Allowing 10 days to elapse after the final injection, the animal was bled and the serum separated. After inactivation at 56° C for 30 minutes, the sera were stored at —60° C. No animal died during the immunization.

a) Precipitation

The immune serum was tested by the precipitin test (ring-method) against the ME and the conidial extract (CE). Conidial extract was prepared as follows: 20 loopfuls of conidia were suspended in 1 ml of saline and repeated cryolysis was performed as in the case of the suspension of dried mycelia. The suspension was then filtrated to eliminate the remaining conidia and the debris.

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[1]) delivered from the Institute for Fermentation (Osaka).

The result is shown in Table I. The immune sera fairly reacted against ME and CE.

TABLE I.

Precipitation of anti-Aspergillus fumigatus serum against homologous conidial and mycelial extracts

Antigen / Antiserum	Conidial extract (Hanshin strain) (undiluted)	Mycelial extract (Hanshin strain)				Saline control
		undiluted	$\times 10$	$\times 10^2$	$\times 10^3$	
Anti-Asp. fumig. (Hanshin strain)	++	++	++	+	—	—
Anti-Asp. fumig. (4040 strain)	++	++	++	+	—	—
normal rabbit serum	—					—

b) Complement Fixation

As test antigens, ME, CE and conidial suspension (0.1 mg/ml) were chosen.

The antigens were tested for their anticomplementary effects prior to their use in the test proper. Except for the CE which was found highly anticomplementary, ME (when diluted 1 : 5) and conidial suspension (diluted more than 1 : 16) could be safely used.

TABLE II.

Procedures of complement fixation test

	column I (serum controls)	column II	antigen control
serum complement (2M.H.D) antigen	0.25 ml to each tubes 0.25 ml to each tubes (saline 0.25 ml)	0.25 ml 0.25 ml 0.25 ml	(saline 0.25 ml) 0.25 ml 0.25 ml
overnight refrigerated			
hemolytic system (7M.H.D sensitized 3 % bovine red cell suspension)	0.25 ml	0.25 ml	0.25 ml
37° C water bath for 30 minutes			
reading			

TABLE III.

Complement fixation of anti-Aspergillus fumigatus serum against homologous mycelial extract (ME) and conidial suspension

serum	antigen	serum dilution							Antigen control
		×40	×80	×160	×300	×640	×1280	×2560	
anti-Asp. fumigatus serum (Hanshin strain)	ME ×140	0	0	0	0	1′	2	3′	3
	ME ×14000	0	0	0	2′	3′	3	3	3
	conidial suspension ×20	2′	2	3′	3	3	3	3	3
	conidial suspension ×2000	2	3	3	3	3	3	3	3
	serum control	3	3						

Technique of the complement fixation test is given in Table II; and Table III shows the result with the anti-Asp. fumigatus (Hanshin strain) serum. 1 : 140 diluted ME gave a positive complement fixation up to the serum dilution of 1 : 2560, while the conidial suspension gave a positive reaction to 1 : 160 dilution of the same serum. Compared with the fact that the hitherto known highest titer of anti-Asp. fumigatus serum was 1 : 20 or 1 : 40, our serum should be rightfully regarded as an unparalled high-titered one.

II. PREPARATION OF IN VITRO ANTIGENS AND THEIR SPECIFICITY

1) Conidial Suspension

In the serology of fungi, suspensions of conidia have been widely used either as immunizing agents or as in vitro antigens. The authors also tested conidial suspensions of various fungi for their reactivity with anti-Asp. fumigatus serum. As it was difficult to prepare a suspension of *Asp. fumigatus* conidia which could be used as the antigen for agglutination or precipitation tests, the authors performed complement fixation tests only.

Saline suspensions (5 mg/ml) of conidia of *Asp. fumigatus* (Hanshin, No. 4040 and No. 4140 strains), *Asp. oryzae* (No. 4038 strain), *Penicillium chrysogenum* (Q 176 strain) and *Actinomyces streptomises* (1408 strain) were prepared by shaking the tubes vigorously. They were used in concentrations exhibiting no anticomplementary effects.

The results are shown in Table IV. Anti-Asp. fumigatus serum gave higher titers against *Penicillium chrysogenum* conidia than against the homologous conidia. Even taking into consideration the difference in concentration of suspension, the reaction to conidial suspension could not be regarded as specific. Moreover, normal rabbit serum reacted to conidial suspension of *Asp. fumigatus* and to its ME to serum titers of 1 : 40 and 1 : 160 respectively. Therefore,

TABLE IV.

Complement fixation of anti-Asp. fumigatus serum against conidial suspensions of various fungi

antigens	dilution of antigen (undiluted: 5 mg/ml)	complement fixing titers of serum
Asp. fumigatus Hanshin strain	× 16	× 80
Asp. fumigatus No. 4040	× 16	× 40
Asp. fumigatus No. 4140	× 16	× 80
Asp. oryzae No. 4038	× 20	× 40
P. chrysogenum Q 176	× 40	× 160
Act. streptomises 1408 A	× 1280	× 80

conidial suspension was found to be inadequate as in vitro antigen in studies on *Asp. fumigatus.*

2) Alcoholic Precipitate Antigen Prepared from ME

As ME of *Asp. fumigatus* reacted with normal rabbit serum to a considerable titer, the authors tried to find a more specific antigen. Alcoholic precipitate of ME was first tested for this purpose. Ethyl alcohol was added to ME of *Asp. fumigatus* (Hanshin and No. 4040 strains) to 65 % and the mixture left in a refrigerator overnight. The precipitate was then separated by centrifugation and dried in vacuo. The resulting grayish white powder slowly dissolved in water to make a viscous fluid. Its 0.1 % (w/v) solution in distilled water gave a positive biuret and Molisch reaction. This fraction hereafter will be called the FI.

a) Precipitation

Asp. fumigatus FI 8 mg was dissolved in 24 ml of physiological saline. Its ten fold dilutions were layered over anti-Asp. fumigatus (Hanshin strain) serum. As given in Table V, this fraction even diluted to 3×10^{-3} γ/ml still gave precipitation with the immune serum.

TABLE V.

Precipitation of Anti-Asp. fumigatus serum against homologous FI

Antigen	serum	Concentration of antigen (γ/ml)				saline control
		3×10^{0}	3×10^{-1}	3×10^{-2}	3×10^{-3}	
Asp. fumigatus (Hanshin) FI	Anti-Asp. fum. (Hanshin) serum	+	+	+	+	—
	normal serum	—	—	—	—	—
Asp. fumigatus (4040) FI	Anti-Asp. fum. (Hanshin) serum	+	+	+	+	
	normal serum	—	—	—	—	

b) *Complement Fixation*

FI antigen, which has not exhibited anticomplementary effect in a concentration of 1500 γ/ml, reacted in 300 γ/ml concentration with the homologous immune serum to 1 : 3200 titer. Normal rabbit serum did not reacted at 1 : 50 dilution (Table VI).

Table VI.

Complement fixation of anti-Asp. fumigatus serum against homologous FI

concentration of antigen (γ/ml)		serum dilution								antigen control
		×50	×100	×200	×400	×800	×1600	×3200	×6400	
Asp. fumig. FI	3×10^2	0	0	0	1′	1	2	3′	3	3
	3×10^1	0	0	0	1′	3′	3	3	3	3
	3×10^0	0	2′	3′	3	3	3	3	3	3
serum control		3	3							

c) *Specificity of FI Antigen*

From mycelial extracts of *Asp. oryzae* (No. 4038), *Candida albicans* (1038) and *Penicillium chrysogenum* (Q 176) prepared by the same procedure of cryolysis as applied to *Asp. fumigatus*, FI antigens were precipitated by alcohol. In the case of *Cryptococcus neoformans*, FI antigen was prepared by alcoholic precipitation of culture fluid.

These FI antigens were tested for their reactivity with anti-Asp. fumigatus, anti-Candida albicans and anti-Cryptococcus neoformans sera.

Table VII.

Precipitation of anti-Asp. fumigatus serum against FI and FII of various fungi.

serum	antigens		antigen concentration (γ/ml)					Control
			1000	100	10	1	0.1	
Anti-Asp. fumigatus (Hanshin strain) serum	Asp. fumigatus (Hanshin strain)	FI	+	+	+	+	+	—
		FII	+	+	+	+	—	
	Asp. fumigatus 4040	FI	+	+	+	+	+	
	Asp. fumigatus 4140	FI	+	+	+	+	+	
	Asp. oryzae No. 4038	FI	+	+	—	—	—	
		FII	+	—	—	—	—	
	P. chrysogenum Q 178	FI	+	+	—	—	—	
		FII	+	—	—	—	—	
	C. albicans 1038	FI	—	—	—	—	—	
		FII	—	—	—	—	—	
	Cr. neoformans	FI	—	—	—	—	—	

TABLE VIII.

Complement fixation of anti-Asp. fumigatus serum against FI and FII of various fungi

serum	antigens 1000 γ/ml		titer of the serum
Anti-Asp. fumigatus (Hanshin strain) serum	Asp. fumigatus (Hanshin strain)	FI	× 2560
		FII	× 1280
	Asp. fumigatus 4040	FI	× 1280
		FII	× 640
	Asp. fumigatus 4140	FI	× 1280
	Asp. oryzae No. 4038	FI	× 320
		FII	× 80
	P. chrysogenum Q 178	FI	× 640
		FII	× 320
	C. albicans 1038	FI	× 80
		FII	× 40
	Cr. neoformans	FI	× 40

Anti-C. albicans serum was prepared by immunizing rabbits with cryolytic extract of *C. albicans* cells cultivated at 37° C for 48 hours in Sabouraud's fluid medium with continuous shaking. Anti-Cr. neoformans serum was obtained by immunization of rabbits with suspensions of *Cr. neoformans* Yamada strain (isolated from a patient in the Psychiatric Clinic of the Osaka University Medical School).

Results:

Tables VII and VIII show the results of precipitation and complement fixation tests of various antigens with anti-Asp. fumigatus serum.

FI antigens from two strains of *Asp. fumigatus* gave precipitation with homologous serum even in the antigen concentration of 0.1 γ/ml. FI antigens of *Asp. oryzae* and *P. chrysogenum* reacted first in the antigen concentration of 100 γ/ml and *C. albicans* and *Cr. neoformans* FI reacted only in a concentration of 1000 γ/ml.

From the results of complement fixation tests, it should be noted that *P. chrysogenum* FI (1000 γ/ml) reacted with anti-Asp. fumigatus serum to the titer of 1 : 640, while the hemologous FI reacted to 1 : 2560.

Table IX shows the result with anti-Cr. neoformans serum. By precipitin tests, only the homologous FI gave a positive result. Complement fixation was observed not only, with *Cr. neoformans* FI (to 1 : 160 serum titre), but also with *Asp. fumigatus* (1 : 80) and other FI antigens (1 : 20).

Table X indicates the result with anti-C. albicans serum. Only the homologous antigens reacted strongly, while all the heterologous

TABLE IX.

Precipitation and complement fixation of anti-Cr. neoformans serum against FI and FII of various fungi

serum	antigens	Precipitation				saline control	complement fixation (antigen 1000 γ/ml)
		antigen concentration (γ/ml)					titer of the serum
		1000	100	10	1		
Anti-Cr. neoformans serum	Chr. neoformans FI	+	+	—	—	—	× 160
	Yamada strain FII	+	—	—	—		× 40
	Asp. fumigatus FI	—	—	—	—		× 80
	Hanshin strain FII	—	—	—	—		× 80
	Asp. oryzae 4038 FI	—	—	—	—		× 40
	C. albicans 1038 FI	—	—	—	—		× 20
	FII	—	—	—	—		× 20
	P. chrysogenum FI	—	—	—	—		× 20

TABLE X.

Precipitation and complement fixation of anti-C. albicans serum against FI and FII of various fungi

serum	antigens	Precipitation				saline control	complement fixation (antigen 1000 γ/ml)
		antigen concentration (γ/ml)					titer of the serum
		1000	100	10	1		
Anti-C. albicans serum	C. albicans 1038 FI	+	+	—	—	—	× 320
	FII	+	+	—	—		× 320
	C. albicans Akiba's antigen	+	+	—	—		× 640
	Asp. fumigatus FI	—	—	—	—		× 20
	Hanshin strain FII	—	—	—	—		× 20
	Asp. fumig. 4040 FI	—	—	—	—		(not tested)
	Asp. oryzae 4038 FI	—	—	—	—		× 20
	P. chrysogenum Q178 FI	—	—	—	—		—
	Cr. neoformans FI	—	—	—	—		—

antigens reacted either weakly or not at all. The reactivity of the precipitation antigen devised by Prof. AKIBA was almost equal to our *C. albicans* FI in the precipitin test and somewhat higher than ours in the complement fixation test.

3) Deproteinated Antigen (FII)

According to the method given in a report by THJØTTA et al. (1951), the authors tried to remove proteins from FI. To 250 ml of 0.1 % FI solution in 0.3 M phosphate buffer (pH 6.0), 50 ml of chloroform and 10 ml of N butanol were added. The mixture was vigorously shaken for 30 minutes, and then centrifuged. The precipitated protein composed a thin layer between the layers of chloroform and water. The precipitate and chloroform were discarded and the watery layer was again similarly treated with chloroform-butanol until no protein layer resulted. To the final aqueous solution ethyl alcohol was added to 65 % and left in a refrigerator overnight. The resulted precipitate was separated by centrifugation and dried in vacuo. This white powder (hereafter called as FII) dissolved slowly in distilled water to make a viscous and milky fluid. With a 0.1 % aqueous solution the Molisch reaction was positive but the biuret reaction negative. So it was thought to be mainly composed of polysaccharides. From *Asp. oryzae, C. albicans* and *P. chrysogenum,* FII antigens were prepared by the same procedures.

Results:

As shown in Table VII, *Asp. fumigatus* FII (1 γ/ml in physiological saline) gave precipitation with homologous serum. By complement fixation test (Tab. VIII) FII gave a somewhat lower titer than FI. Here too, *P. chrysogenum* FII reacted with anti-Asp. fumigatus serum to a considerably high titer.

Anti-Cr. neoformans serum reacted only against the homologous FII by precipitin tests. The specificity of complement fixation test was rather lower than that with FI. Homologous FII have a lower titer (1 : 40) than *Asp. fumigatus* FII. (Tab. IX).

With anti-C. albicans serum, only the homologous FII strongly reacted (Tab. X).

Thus, concerning the three pathogenic fungi (*Asp. fumigatus, C. albicans* and *Cr. neoformans*) there was observed no marked difference in antigenic activities between the FI and FII.

III. SKIN TEST IN EXPERIMENTALLY INFECTED ANIMALS

1) Guinea Pigs

Two groups of guinea pigs A and B, each consisting of 3 animals were used. *Asp. fumigatus* conidia (Hanshin strain) was inoculated intraperitoneally, 0.5 mg to each animal of A group, 0.1 mg to each of the B group. No animal died during the experiment. 14 days after the inoculation 0.1 ml each of *Asp. fumigatus* FI

(1000 γ/ml), *P. chrysogenum* FI (1000 γ/ml), *Cr. neoformans* FI (1000 γ/ml) and physiological saline were injected intradermally into four sites on the shaved abdominal wall. Readings were taken 24 hours and 48 hours after the injection of antigens. Redness larger than 5 mm in diameter was interpreted as positive.

All the animals showed positive reactions at the injection site of *Asp. fumigatus* FI.

No difference in the degree of redness was observed between the animals of A and B groups. By *P. chrysogenum* FI, only one animal showed a positive reaction at the 24 hours' reading, while at the 48 hours' reading no positive reaction was observed. *Cr. neoformans* FI gave no reaction in any of the animals.

2) Rabbits

Conidia of *Asp. fumigatus* (Hanshin strain) cultivated for 7 days on Czapek's agar medium were inoculated to 5 rabbits intravenously (0.3 mg/kg). Two rabbits succumbed during the experiment. As controls 2 rabbits immunized with *C. albicans*, 1 with *Pullularia pullulans* and 2 normal rabbits were used in this experiment.

The test antigens used were: *Asp fumigatus* (Hanshin strain) FI and FII, *P. chrysogenum* FI, *Cr. neoformans* FI, *C. albicans* skin test antigen devised by Prof. AKIBA, and saline control.

First, various concentrations of *Asp. fumigatus* FI and FII were tested for their reactivity. Each 0.1 ml of FI (1000 γ/ml, 100 γ/ml and 10 γ/ml) and FII (500 γ/ml, 50 γ/ml and 5 γ/ml) were injected into six sites on the shaved abdominal wall intradermally. Reading

TABLE XI.

Skin test of Asp. fumigatus infected rabbits with various concentrations of homologous FI and FII

	Rabbit		Asp. fumigatus FI (γ/ml)			Asp. fum. FII (γ/ml)		
	No.	days after inoculation	1000	100	10	500	50	5
24 hours' reading	No. 5	7	5mm × 5mm	5 × 5	0	8 × 7	0	0
	No. 4	15	10 × 8	5 × 5	4 × 5	7 × 7	3 × 5	0
	No. 6	40	5 × 5	3 × 3	0	5 × 5	0	0
	immunized with C. alb.		3 × 3	0	0	2 × 3	0	0
	normal rabbit		0	0	0	0	0	0
48 hours' reading	No. 5	7	8 × 10	5 × 5	0	5 × 5	0	0
	No. 4	15	10 × 10	10 × 10	8 × 8	10 × 10	5 × 5	0
	No. 6	40	10 × 10	5 × 5	0	8 × 7	0	0
	immunized with C. alb.		0	0	0	0	0	0
	normal rabbit		0	0	0	0	0	0

was performed just as in the cases of guinea pigs. As can be seen from the results given in Table XI, it was found that optimal concentrations for skin test were 100 γ/ml for FI and 50 γ/ml for FII, and that 24 hours seemed too short a period to exclude para-reactions as was observed in a control rabbit immunized with *C. albicans*.

Then we carried out skin test on 4 rabbits infected with *Asp. fumigatus* with *Asp. fumigatus* FI (100 γ/ml) and FII (50 γ/ml), *P. chrysogenum* FI (1000 γ/ml), *Cr. neoformans* FI (1000 γ/ml) and AKIBA's *C. albicans* antigen (5 γ/ml). Final reading was taken 36 hours after the injection of antigens. As shown in Table XII, no reaction was observed by injection of *C. albicans* or *Cr. neoformans* antigens. By *P. chrysogenum* FI, slight redness was observed in two animals, which could not be interpreted as positive.

TABLE XII.

Skin test of Asp. fumigatus infected rabbits with various fungous antigens (36 *hour's reading*)

Rabbit		antigens				
No.	days after inoculation	Asp. fumig. FI 100 γ/ml	Asp. fumig. FII 50 γ/ml	P. chrys. FI 1000 γ/ml	C. alb. Akiba's antigen 5 γ/ml	Cr. neof. FI 1000 γ/ml
5	21	15^{mm} × 20^{mm}	15 × 15	0	0	0
3[1]	21	15 × 15	5 × 5	2 × 2	0	0
7	29	15 × 15	10 × 10	2 × 3	0	0
6	(29 after reinoculation)	20 × 15	15 × 15	0	0	0
immunized with C. albicans		2 × 2	2 × 2	0	5 × 6	0
,, P. pullulans		0	0	0	0	0
normal rabbit		0	0	0	0	0

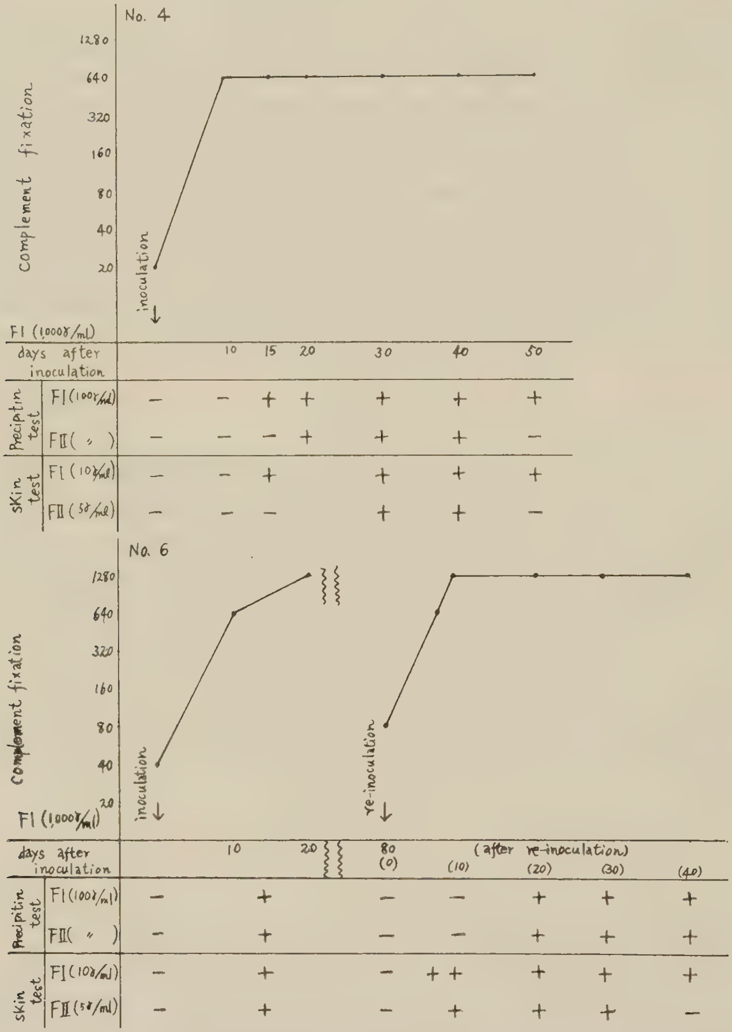
[1] died during the experiment.

A control rabbit immunized with *C. albicans* slightly reacted to *Asp. fumigatus* FI and FII, but the reaction soon subsided. A normal rabbit and a rabbit immunized with *Pullularia pullulans* reacted against none of the antigens used.

IV. CORRELATION OF SEROLOGICAL AND SKIN TEST RESULTS

Five rabbits inoculated with *Asp. fumigatus* conidia were checked by precipitation, complement fixation and skin tests to find the trend of the three reactions during the course of infection. Examples of the results are given in Fig. 1.

In rabbit No. 4 (Fig. 1), precipitin test turned to positive from the 15th (FI) to the 20th (FII) day, while there was a marked rise in complement fixing titer observed on the 9th day. Skin test became positive on the 15th day.



No. 4

days after inoculation		10	15	20	30	40	50
Precipitin test F I (100γ/ml)	−	−	+	+	+	+	+
Precipitin test F II (〃)	−	−	−	+	+	+	−
Skin test F I (10γ/ml)	−	−	+		+	+	+
Skin test F II (5γ/ml)	−	−	−		+	+	−

No. 6

days after inoculation		10		20	80 (0)	(after re-inoculation) (10)	(20)	(30)	(40)
Precipitin test F I (100γ/ml)	−		+		−	−	+	+	+
Precipitin test F II (〃)	−		+		−	−	+	+	+
Skin test F I (10γ/ml)	−		+		−	+ +	+	+	+
Skin test F II (5γ/ml)	−		+		−	+	+	+	−

Fig. 1. Serological and skin test results in the rabbits inoculated with *Asp. fumigatus* conidia. In the skintest 100 γ/ml were used for the F I and 50 γ/ml for the F II, instead of the 10 γ and 5 γ as indicated in the figure.

In rabbit No. 6 (Fig. 1), all the three tests were positive on the 15th day. On the 80th day the complement fixation test alone remained positive (1 : 80), while the other two reactions had already turned to negative. When *Asp. fumigatus* conidia was reinoculated to this rabbit, the complement fixation test responded with a marked rise in titer on the 7th day after the reinoculation. Skin test also became positive again on the 7th day, while precipitin test still remained negative on the 10th day after the reinoculation.

Summarizing the results in all the rabbits tested, it can be said that the earliest response to the infection was the positive complement fixation test (or marked rise in its titer) followed by skin test and precipitin test. Moreover, both in precipitation test and in skin test FI reacted more sensitively than FII.

V. DISCUSSION

The greatest difficulty to be met with in the serology of fungi is the obtaining of high titer immune serum. Especially with *Asp. fumigatus*, no one has yet succeeded in immunization, not only because of the difficulty in raising the titer but also because of too frequent loss of animals during the immunization.

In the experiment of MATSUMOTO, all the rabbits immunized with suspensions of *Asp. fumigatus* conidia succumbed in the first few days of the immunization course leaving sera with a complement fixation titer less than 1 : 10.

In the report of SALVIN, who studied the specificity of immune sera against various fungi, the complement fixing titer of anti-Asp. fumigatus sera was 1 : 40. It seems to us that one can hardly study specificity with such a low titer serum.

The authors turned their attention to the possibility of obtaining high titer immune serum by employing extract of *Asp. fumigatus* mycelia instead of intact conidia as the immunizing agent. They collected mycelia without developing conidia through shaking culture. Then, they tried to disintegrate the cells by repeated cryolysis, which had been known to them to be effective in obtaining a toxic extract from the pellicle of the same mold. By this method the authors were able to obtain immune serum with high precipitin and complement fixation titers.

The use of fungi extract obtained by repeated cryolysis as the immunizing agent had been already tried formerly with a kind of *Rhizopus*. In the report by NEILL et al., it was given that they used as immunizing agents the cryolytic extracts of several fungi including *Aspergillus amstelodami*. Based on the results of comparative studies on the methods of preparing immunizing and in vitro antigens from fungi, THJØTTA et al. recommended cryolytic extracts instead of intact cells for immunization. Taking these reports into consideration, the authors' idea of the preparation of immunizing antigen should be said to be in accord with the trend of recent times.

Then, the authors chose alcoholic precipitate of ME (FI) and the fraction obtained from FI by chloroform-butanol treatment (FII) as antigens in vitro and studied their specificity.

Conidial suspension of *Asp. fumigatus* of course reacted with our anti-Asp. fumigatus serum. However its reactivity was far behind that of ME and was no better than its reaction with normal rabbit serum. Thus, the conidial suspension was excluded from our studies.

The reactivity of *Asp. fumigatus* FI with the homologous immune serum was found to be satisfactory. Its specificity was also clearly demonstrated especially by the precipitin test, employing anti-C. albicans and anti-Cr. neoformans sera as controls. Moreover, there was observed no marked difference in the antigenic activity between FI and FII.

Till recently, suspensions of spores had been exclusively used as in vitro antigens in the serology of fungi. Now, they are gradually replaced by soluble antigens except in the cases of some yeast-like fungi where the cell suspensions are still used by some authors for limited purposes.

Supernates of 6-8 week old cultures of Aspergilli were first used by MATSUMOTO as antigens in complement fixation test. Although culture fluids or antigens purified from them are widely used in the serology of encapsulated fungi as Cr. *neoformans*, no one but MATSUMOTO applied culture fluid of Aspergilli to serological tests.

Another method of obtaining soluble antigen is to disintegrate fungi cells and fractionate the resulted extracts. HENRICI observed feeble precipitation of *Asp. fumigatus* cell sap with the sera of previously infected animals. DRAKE also noticed that the cell sap of *Asp. nidulans* reacted with the sera of rabbits injected with the homologous mold. The antigenic activity of a crude polysaccharide fraction prepared from *Asp. fumigatus* mycelia was studied by STANLEY by serological and skin tests.

Thus, it can be said that either started from culture fluid or from extract of fungi cells, the attention of recent workers studying fungous soluble antigens seems to be concentrated to the significance of polysaccharide fraction. Although there are varieties in the procedures of fractionation due to the purposes of the experiments or to the different properties of the fungi used, it seems to us that fairly specific antigens have been obtained by alcoholic precipitation in many cases, as by NEILL et al. from *Cr. neoformans* and *Sporotrichum schenckii*, or by THJØTTA et al. from *Geotrichum* species and *C. albicans*. Especially it should be noted that the procedures employed by THJØTTA et al. are identical to ours, in as much as they started from cryolytic extracts and that they further treated alcoholic precipitate with chloroform-butanol. Therefore, the authors' results with *Asp. fumigatus* and several other pathogenic fungi will serve as an extension from and confirmation of the statements by THJØTTA et al. in the discussion how to standardize the methods of preparing fungous soluble antigens. In STANLEY's report, the procedures of fractionation are somewhat different to ours and the specificity of antigens has not been fully studied. However, his results are similar to ours in the facts that the fraction mainly composed of polysaccharides has been found active as serological and skin test antigens.

Although it seems no time to preclude what kind of antigen should be used in future for the systemic taxonomy of fungi, we can at least point out in concern to *Asp. fumigatus* the following three facts from the results of our experiments:

a) FI and FII are superior to conidial suspension in specificity;
b) No marked difference is found in the antigenicity between FI and FII;
c) Considerable para-reactions are observed between *Asp. fumigatus* and *P. chrysogenum* by complement fixation tests, even when FI or FII antigen is used.

Skin reaction in the guinea pig and rabbit inoculated with *Asp. fumigatus* conidia against homologous FI and FII can be interpreted as the delayed type allergy. Both FI and FII were found specific enough to be used as skin test antigens when the final reading was taken 36 hours after the injection of antigens, although somewhat better results were obtained with FI than with FII.

Concerning the diagnostic value of fungous skin test antigens, many workers have been rather critical. Here too, cell suspensions or crude culture filtrates of older days are now replaced by soluble antigens fractionated by alcoholic precipitation or other procedures. For example, in their comparative studies of skin test antigens with the rabbits infected with *Blastomyces dermatitidis*, DYSON et al. reported that the alcoholic precipitate antigen gave the best results. Moreover, they share common findings with us in that antigens obtained by further treatment with chloroform was rather inferior to the alcoholic precipitate.

Thus, it can be concluded that the immunizing, serological and intradermal antigens employed by the authors have all been with the recent trend in the serology of fungi. When the authors started the experiments no one paid attention to the significance of serological reactions in the study of mycoses. At that time many of the works abroad listed in the references had been either unavailable or not rightly estimated by researchers in this country. Since then, increasing case reports of aspergillosis in man have arrested the attention of physicians. For example, many cases of pulmonary aspergillosis, clinically resembling tuberculosis were reported among the laborers handling chicken feed by MIYANO. From the autopsy statistics (1946-1954) in the Department of Pathology, Tokyo University Medical School, it was found that aspergillosis (15 cases) was most frequently met with among the mycoses, surpassing the candidiasis (2 cases). Yet, at present, the physicians must rely merely on the repeated isolation of *Asp. fumigatus* from specimens of sputum and on the characteristic X-ray findings. Therefore, the establishment of reliable immunological diagnostic measures is greatly waited for. It is due to the same reason that many workers are trying to prepare diagnostic antigens for candidiasis and other mycotic infections. The authors would be happy if their results with *Asp. fumigatus* would be of some help to the researchers who are engaged in the serology of mycoses.

SUMMARY

1) Extract was obtained by repeated cryolysis of acetone-dried

mycelia of *Asp. fumigatus* killed by formalin. High titer immune serum could be easily obtained by immunizing rabbits with this extract.

2) FI antigen prepared from mycelial extract by alcoholic precipitation exhibited species-specificity when examined by the precipitin test.

3) FII, prepared from FI by chloroform-butanol treatment, was no better than FI in antigenic activity.

4) FI could be used as antigen in the skin test with rabbits infected with *Asp. fumigatus*, when the final reading was based on the redness (larger than 5 mm in diameter) 36 hours after the injection of antigen.

ACKNOWLEDGEMENT

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TREATMENT OF EXPERIMENTAL SPOROTRICHOSIS IN MICE

by

E. Tsubura & J. Schwarz [1])

(9 VIII. 1960)

In a previous study (1), treatment of experimental sporotrichosis was reported after oral intake of griseofulvin and intraperitoneal injection of Amphotericin B. In view of the poor results it was assumed that possibly the infecting dose was so overwhelming that no therapeutic effect could be found, and a new series was started adding to the therapeutic trials Trichomycin and Potassium Iodide.

Materials and Methods

2.3×10^5 cells of a saline suspension of *Sporotrichum schenckii*, (from the culture collection of Dr. Norman F. Conant, Duke University, Durham, North Carolina) which were grown in the yeast phase on blood agar at 37° C for three days were injected intravenously into female white Swiss mice, weighing approximately 18 g. Twenty-four hours after inoculation, the mice were arbitrarily divided into groups of 25 each and the following treatment schedules started:

1. Griseofulvin was incorporated into the food, resulting in an intake of 75 to 100 mg/kg/day.
2. Amphotericin was injected intraperitoneally at a level of 1 mg/kg/day for two consecutive weeks.
3. Trichomycin was dissolved in 1 normal NaOH adjusted to a pH of 7.0 with normal HCl and injected intraperitoneally in a concentration of 0.5 mg/kg/day 6 times within 10 days leaving some days without treatment in order to avoid toxicity.
4. Potassium Iodide was prepared in saturated solution and given to mice in the drinking water. This was continued until spontaneous death occurred. The daily dosage was 0.6-0.8 g/kg.

Cultures were taken from liver and spleen of all animals and the organs were examined for the presence of inflammatory response.

The result of the treatment is shown in Table I.

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[1]) From the Clinical Laboratories, Jewish Hospital, Cincinnati, Ohio.

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TABLE I.

Treatment of experimental sporotrichosis in mice

Group	Mortality	Positive Culture results from Liver and Spleen
I. Untreated [1]) control	25/25	25/25
II. Griseofulvin orally 100 mg/kg/day	25/25	25/25
III. Potassium Iodide, orally 0.62-0.8 g/kg/day	25/25	25/25
IV. Amphotericin B, intraperitoneally 1 mg/kg/day for 2 weeks	25/25	25/25
V. Trichomycin [1]) intraperitoneally 0.5 mg/kg/day for 6 times	25/25	25/25

[1]) Groups I and V were injected with 7.5×10^5 cells of the organism, all others with 2.3×10^5.

DISCUSSION

Intravenous injection of *Sporotrichum schenckii* produces disseminated disease which experimentally cannot be easily influenced by the available therapeutic measures. Only Amphotericin B in high dosage can prevent death and can produce sterilization of tissue (1). 1.0 mg/kg/day of Amphotericin B, the dose generally employed in human cases, is not effective in the control of murine infection, and neither griseofulvin nor trichomycin prolong the life of the animals. The failure of KI to influence the course of experimental sporotrichosis is remarkable in view of its apparent effect in the treatment of human infection.

SUMMARY

Griseofulvin, Amphotericin B, Trichomycin and Potassium Iodide did not influence the course of experimental sporotrichosis with an infective dose of 2.3×10^5 cells injected intravenously.

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TRICHOPHYTON VIOLACEUM INFECTION OF THE SCALP IN POONA (INDIA)

by

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(with 5 figs)

(26.VII.1960)

Trichophyton violaceum Sab: apud Bodin, is widely distributed, being a common incitant of ringworm of the scalp, and rarely of beard, skin and nails. It has been reported from various European countries including Italy, France, Russia and Poland. Report from Israel on tinea capitis revealed that a good proportion of the cases were due to *T. violaceum* (Raubitschek 1956). In Central Africa it is one of the common organisms inciting scalp infection (Vanbreuseghem 1957a). This organism is not unknown in Australia, and in U.S.A. sporadic outbreaks have been reported (Rosenthal et al. 1958).

In India, *T. violaceum* may prove to be the chief incitant of scalp infection in majority of cases. It was isolated as early as 1929 by Acton & McGuire (1929) and later pointed out to be the common scalp infecting organism in Calcutta by Dey & Maplestone (1935). Recent reports from Delhi (Behl & Sharma 1957) and Bombay (Desai et al. 1957) have confirmed its predominance in inciting scalp infection. In the present study of scalp infections in Poona, it has been found that *T. violaceum* is the causal organism in 85% of the cases. This report is an account of studies on thirty-four cases of infection incited by *Trichophyton violaceum.*

MATERIAL AND METHODS

Material: Twenty-eight out of thirty-four cases under report were from "Remand Home for Boys — District Probation and After Care Association Poona". Six other cases were patients from different localities of the city. All the cases were males, their age ranging from 5 to 14 years.

Methods: In each case the causative pathogen was identified by direct microscopic examination as well as by isolation in artificial culture. For microscopic examination, the epilated infected hairs from the lesions were mounted in 10 % KOH solution. These preparations revealed endothrix type of infection invading the hair.

Artificial culture of the causative organism was made from the affected hairs, which were inoculated on Sabouraud's dextrose agar and incubated at 28° C. These were examined periodically, and were discarded as negative only when incubation even after four weeks gave negative results.

HISTORY

A large majority of cases of scalp infection are presented in our department during the months of February to June. It is observed that there is a considerable decline in the number of cases after the monsoon rains set in from June to September.

The source of initial infection in Remand Home could not be traced. The disease attracted the attention of the authorities when eighteen inmates complained of intense itching of the scalp. By March, the number of cases had increased to twenty-eight. Six cases of the other group came in during the same period and were detected during routine examinations. Places of residence of these patients were well-spread all over the city.

All the 34 cases were male patients, their age ranging from 5 to 14

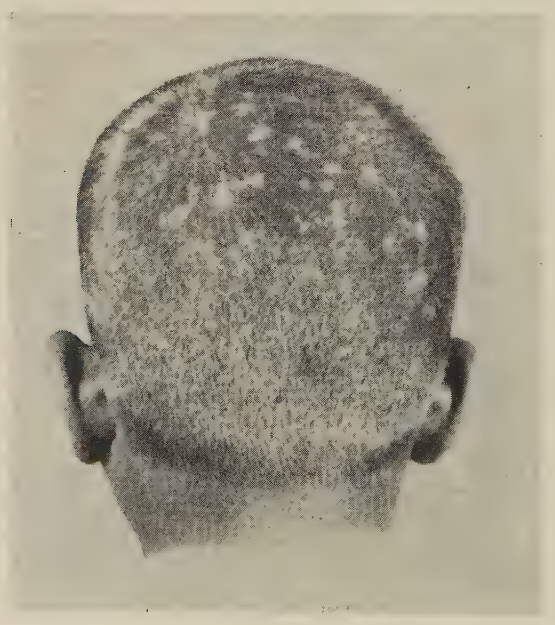

Fig. 1. Scaly encrusted patches

years. They belonged to low income group and majority of them manifested clinical signs of malnutrition. It became apparent that epidemic in Remand Home was due to close contact between children in institutional life; in addition to negligence of personal hygiene. Infection in all cases was restricted to the scalp only and in no case the organism could be recovered from other parts of the body.

On the basis of symptomology, the cases under study were divided into 5 clinical types (1) Scaly crusted superficial patches (Fig. 1), (2) Black dot appearance (Fig. 2), (3) Pustular inflammatory lesions (Fig. 3), (4) Kerion (Fig. 4), (5) Partial alopecia (Fig. 5).

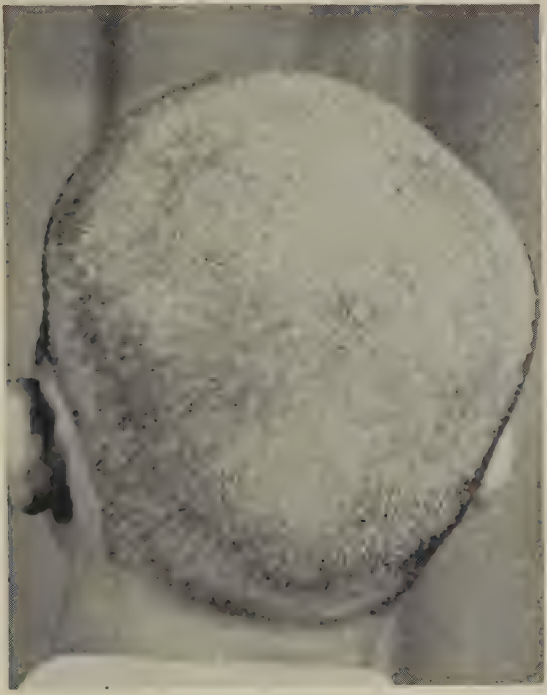

Fig. 2. Black dot appearance.

1) Scaly crusted superficial patches:

This clinical type was the commonest manifestation seen. Eighteen cases investigated presented this picture. The scales were greyish white, adherent to the hair at the level of the scalp. The patches appeared as irregularly-contoured areas over the scalp. Hair from these areas were relatively sparse, dry, without lustre and could be epilated easily. Microscopic examination of the hair revealed endothrix infection.

2) Black dot appearance:

This classical clinical type presented only in seven cases. Scaly crusts were markedly absent. Infected hairs thinned out and became broken at the surface or little above the scalp near the mouth of the hair follicle leaving stumps of the hair behind and thus giving typical "black dot" appearance.

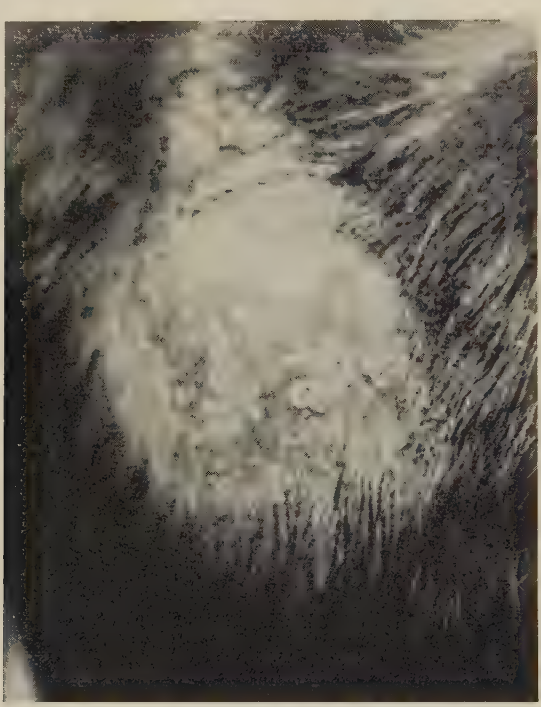

Fig. 3. Pustular inflammatory lesions.

3) Pustular inflammatory lesions:

Active inflammation and pustule formation in the follicles which is described as a common condition in Italy by DELLA FAVERA (1909) was observed in six patients. In these cases, some small bald patches measuring 1—2 cm in diameter were also seen in addition to the pustular lesions.

4) Kerion:

During last two years we have observed 8 cases of kerion. In 6 cases of kerion the causative organisms isolated were either *M. canis* or *M. gypseum*. But two cases of kerion in the present study yielded *T. violaceum* colonies in culture.

5) Partial Alopecia:

Castellani (1934) described a clinical type caused by *T. violaceum*, which showed pityriasic scaliness and permanent baldness. This clinical picture was observed in only one case. There were no scaly patches but partial alopecia was observed. Clinically this was thought to be a case of nonmycotic alopecia; but microscopic and cultural examination of the hair from borders of the bald patches revealed *T. violaceum* as the causal organism.

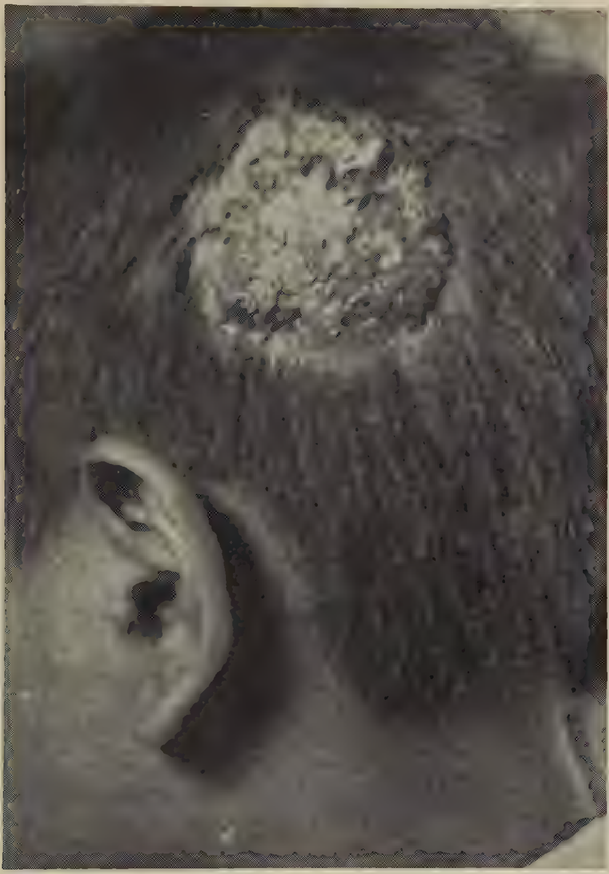

Fig. 4. Kerion formation.

Mycological studies: (A) Microscopic examination of the infected hair was made from mounts in KOH (10 %) solution. Examination after slightly heating the slide showed chains of anthrospores measuring 2—4 μ in diameter, inside the hair-shaft. The spores were oval or slightly oblong, brownish black in colour.

B) *Artificial culture*: The infected hairs were inoculated on Sabouraud's dextrose agar and incubated at 28° C. The colonies of *T. violaceum* developed after 9—16 days. Colonies at first were small, moist glabrous or fluffy-white to grayish in colour, heaped or flat which later developed a lavender to deep purplish pigment. Microscopic examination revealed a mass of racquet hyphae. The only other structures that were seen were chlamydospores.

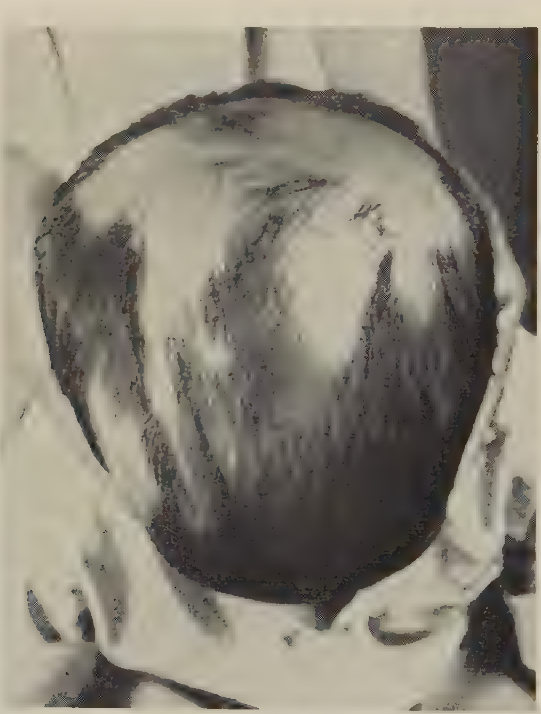

Fig. 5. Incitement of alopecia.

DISCUSSIONS

It is thus evident that the problem of ringworm infection of the scalp incited by *T. violaceum* needs a careful study. Among many factors suggested for spread of tinea capitis, misery and living in non-hygienic conditions has been pointed out as a favouring factor. But as yet no one has indicated the real nature of the predisposing factor. VANBREUSEGHEM (1957a and 1957b) has pointed out that one of the factors may be the protein deficiency or avitaminosis. Our clinical observations are in agreement with these views, but it is likely that a further study of other conditions such as social or economic status of the affected persons, population density, study of personal habits will have to be considered in more detail.

Trichophyton violaceum with its wide geographical distribution is dominant among the scalp infecting fungi in our country. It is quite resistant to classical methods of treatment by anti-fungal medicaments and is well known for its contagious nature. It is therefore felt that a large-scale survey of scalp infections based upon isolation of the causal organism is necessary to understand the epidemiology of *T. violaceum.*

SUMMARY

A report of thirty-four cases of ringworm of the scalp caused by *T. violaceum* studied at Sassoon Hospitals, Poona, India, is presented. The infections were found to be more common in children of the ages between 5 to 15 during the months of February to June. Majority of the affected individuals were undernourished. Symptomatologically the cases fall into five groups.

ACKNOWLEDGEMENT

Authors wish to express their thanks to:

1) Authorities of „Remand Home for Boys, District Probation and After Care Association, Poona".

2) Authorities of Sassoon Hospitals;

3) Authorities of Hindustan Antibiotics Ltd., Pimpri.

We express our deep gratitude to Mr. B. G. GHATE whose co-operation made this investigation possible.

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ABOUT THE NOTE BY R. CIFERRI ON the „PRECIS DE MYCOLOGIE" 2d edition by M. LANGERON and R. VANBREUSEGHEM. [1]

(8.VIII. 1960)

Contrarily to what is expressed in the last note of Professor R. Ciferri about the „Precis de Mycologie by the late M. Langeron" (Mycopathologia et Mycologia applicata" (1960) 12 : 3, 251-253), the authors of the book are, M. Langeron and R. Vanbreuseghem and not M. Langeron. Moreover the second edition of this book has been prepared entirely by myself after the death of my master. There is no doubt that I was entirely responsible for the third section of this book. This fact is evident 1°) from the presentation. The title of the page 449 runs as follows:

„Troisième partie. Mycologie Médicale
par
R.Vanbreuseghem"

2°) from the introduction to the second edition:

> . . .„La première édition comportait onze chapitres. Je les ai répartis en trois parties: Mycologie générale, Techniques et Mycologie médicale. La mycologie générale est essentiellement basée sur la matière de la première édition, mais la disposition de cette matière a été considérablement modifiée. Elle a reçu quelques compléments tirés des notes du Dr. Langeron et de mes lectures. La deuxième partie, consacrée aux techniques, suit le plan adopté précédemment, mais a été complètée par des techniques nouvelles parues depuis 1945. De la troisième partie ou mycologie médicale, je prends l'entière responsabilité. Ce sujet se réduisait, dans la première édition, au chapitre IX, intitulé „A quoi se ramène la mycologie médicale?" Il ne faut voir dans ce titre qu'une boutade, en même temps qu'un désir du Dr. Langeron de mettre en garde le lecteur contre ceux qui sont toujours prêts à créer de nouvelles mycoses sur la foi d'une quelconque moisissure isolée d'un cas troublant. „Lorsque, me disait le Dr. Langeron, on ne sait plus à quel saint se vouer, on pense aux champignons." J'ai complètement supprimé ce chapitre IX et je l'ai remplacé par une troisième partie, entièrement neuve, dans laquelle les mycoses sont étudiées suivant l'ordre alphabétique. Elle comporte dix-sept chapitres. Je me suis efforcé de ne rien omettre d'essentiel, même des travaux les plus récents et j'ai, chaque fois que je l'ai pu, exposé ce que l'on sait de la mycologie animale" . . .

Consequently if the criticism made by Pr. R. Ciferri are correct they refer to myself and not to the late Dr. Maurice Langeron.

R. Vanbreuseghem

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[1] Editor's note. With this reply of Doctor Vanbreuseghem the discussion on this topic is closed.

LIBRI NOVI

S. C. Prescott & C. G. Dunn: *Industrial Microbiology.* Third Edition. McGraw-Hill, New York, 1959, 945 pp., $17.50.

The first three editions of this book appeared in 1940, 1949 and 1959, respectively. Each edition contained notable changes to render the material current. Several new chapters appear in the third edition, including those on steroid transformation, production of amino acids, gibberellic acid and gibberellins, mushroom mycelium, vitamin B-12, dextran, ustilagic acid, yeast enzymes and the biochemical engineering aspects of industrial microbiology. In addition to the new chapters, several others were rewritten to include recent data. The authors state their purpose to be ,,to outline, in a concise but comprehensive manner, the fundamentals of industrial microbiology and to present descriptions of the more important processes within the field''. To a great extent they have achieved this purpose, however, there are certain shortcomings. As is to be expected, the book is several years out of date with respect to industrial developments for obvious reasons. An example is the discussion of vitamin B-12 which is incomplete or outdated, and does not reflect the present technology of industry. Nevertheless, certain improvements could be suggested for forthcoming revisions. For example, the chapter on the acetone-butanol fermentation comprises 35 pages, a reduction of only 5 pages over the second edition, but further reduction would seem to be advisable in view of the fact that this fermentation is not a prominent one in modern industry. The same is true of the acetone-ethanol, 2,3-butanediol, butanol-isopropanol, mold lactic acid, fat, propionic acid and other fermentations. Space devoted to these obsolete processes is anachronous compared to the few pages devoted to the huge antibiotic industry. Moreover, although Prescott & Dunn included quite a number of processes of academic interest only, they failed to include a discussion of recent work regarding the large scale growth of certain algae, notably *Chlorella*, and its possible future significance in industrial microbiology. In general, this book is comprehensive, and probably is closer to the realities and actualities of industry than any other book upon the subject.

The treatment of riboflavin was enlarged and reflects the prominent position of this fermentation in the industry today. About two pages are devoted to the recently developed beta-carotene fermentation. While only 4 antibiotics were discussed in the second edition, there are 16 antibiotics treated, some very briefly, in the new edition. However, the entire section comprises only 73 pages which is a reduction from 100 pages in the second edition. A noteworthy omission among the antibiotics is bacitracin, which is mentioned only in passing. The chapter on mold enzymes was enlarged from 11 pages to 17 to include the new developments in this increasingly prominent field.

Several other omissions are worthy of note. It would seem that a discussion of the techniques of strain maintenance, of screening programs, of isolation of strains, and of strain improvement should be presented in connection with each process, but yet only in a few instances were such subjects included. Probably the most serious omission is the complete absence, except for certain antibiotics, of a presentation of the methods of assay or other procedures used to determine the yields of various desired products. Nor is there any discussion of procedures in isolation and purification of such products, a task which often falls to the microbiologist in modern industry. The use and application of precursors is mentioned briefly in connection with antibiotics, but nowhere else.

On page 370 is found the statement that the manufacture of dextran results from the collaboration of microbiologists, biochemists, chemists, chemical engineers and others. Nowhere is the realism presented that, in fact, every fermentation product in modern industry is the result of such teamwork.

The text is practically free from printing errors, but they do occur. For example, in Table 175, page 648, we find ,,*Pleutotus ostreatus*''. A more glaring error is to be found on page 619, where the following statement occurs,''... *Gibberella fujikuroi* (SAWADA) WOLLENWEBER (the conidial stage of *Fusarium moniliforme* SHELD.)...''.

References to each chapter are divided according to subject. Excellent charts and tables are provided, especially those regarding taxonomy of the yeasts. The book is a must for the bookshelf of the individual actively interested in fermentations and pharmaceuticals, whether he be biochemist, chemist, bacteriologist, mycologist or chemical engineer. The book is well written, illustrative and with an excellent survey of the literature, notably the patent literature, which is so often neglected by workers in academic pursuits.

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Christensen, Clyde M. Common Fleshy Fungi. Second printing Burgess Publishing Co., Minneapolis, Minn., 1959, pp. vii, 246, with 9 plates. US $ 3.-

The new edition represents a substantial revision of the treatment of the first edition, 1946, as is indicated by the enlargement of the book from 45 to 246 pages. Much of the enlargement is due to the inclusion of descriptions of almost every genus and species treated. As in the earlier edition, keys are presented to the families, genera and species of the included fungi. As the title suggests, only the common species are included in the treatment of the following groups of Basidiomycetes: Agaricaceae, Polyporaceae, Hydnaceae, Boletaceae, Clavariaceae, Lycoperdaceae, Sclerodermataceae, Secotiaceae, Nidulariaceae, Phallaceae and Tremellales. Some of the larger Ascomycetes are also treated briefly, including certain Pezizaceae, Helvellaceae and Geoglossaceae, as well as *Xylaria*, *Cordyceps*, *Daldinia* and *Dibotryon.*

There are some inconsistencies, such as the omission of a key to the species of *Lenzites*, the omission of *Calocera cornea* from the key on page 214 but the inclusion of a description of that species, the inclusion of *Tremella albida* in the key on page 214 but omission of a description for that species and the inclusion of *Sarcoscypha coccinea* and *Urnula craterium* under the title ,,Description of species of Peziza."

There are a few printing errors including: Tremallales, (p. iii), *Dictyophora duppicata*, (p. 212), and the omission of a page citation for *Favolus canadensis* in the key on page 152. Also, it would appear that the ,,Key to Species of Boletaceae" on page 188 should read ,,Key to Genera of Boletaceae",

The plates and glossary present the same material as in the first edition and the keys remain unchanged. However, there are included in the text 4 families of Basidiomycetes which were omitted from the earlier edition. An index to genera and species is now included and this adds to the convenience of the manual. Another added convenience for use in the field is the reduction in size of the book, from approximately 8½ × 10½ inches in the 1946 edition to 5½ × 8½ inches. The manual has great utility and will serve the needs of students of fleshy fungi. It is especially recommended for beginning students.

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CIFERRI, R., „*Manuale di Micologia Medica*". Tomo II. II Edizione. Casa Editrice Renzo Cortina, Pavia, 1960. 796 pages, 605 figs., 15 color plates.

This volume completes „Manuale di Micologia Medica" which is presented as the second edition of „Introduzione alla Micologia Medica." It describes systematically 23 mycoses under the headings: definition, geographic distribution, hosts, etiology, sources of infection, symptomatology, examination of pathological material, culture (when applicable), pathology, experimental infection (when applicable), immunology, prognosis, and therapy. Several minor mycoses and mycotic allergies are discussed more briefly. Whereas the first volume presented background mycology and technics, Vol. 2 is devoted to the pathogenic fungi and is intended for the medical mycologist, the graduate and research student, the physician and the technician.

The book is printed on good quality paper and is attractively bound. Many sections are set in small type but are clear and legible. The bibliography (178 pages) follows the text but is divided into alphabetized sections according to chapter. A good subject index for this and the preceding companion volume occupies 72 pages.

Prof. CIFERRI's interest, competence and leadership in systematic mycology are reflected in his extensive historical reviews of proposed taxonomic systems and in his assignment of several Fungi Imperfecti which cause human disease to positions indicating possible relationships to other groups which are better known taxonomically. Not all mycologists will agree with his concept of close relationships between *Coccidioides* and *Rhinosporidium* and between *Paracoccidioides* and *Basidiobolus*. Several of the binomials he uses are not generally accepted but the validity of some of these names is equivocal and the choice rests upon the judgment of the user. However, CIFERRI's relegation of *Sporotrichum schenckii* to the synonymy of *Rhinocladium beurmannii* does not appear to have any validity from the authority of either priority or present usage. The specific name *schenckii* used by HEKTOEN & PERKINS in 1900 clearly has priority. The American strains are deeply pigmented when first isolated and the fact that strains sent to France had already lost some of their fertility and pigmentation led French mycologists to the erroneous conclusion that strains isolated in France differed from *S. schenckii*. This early loss of pigmentation should also receive more critical consideration by those mycologists who would remove this fungus to the Dematiacious genus *Rhinocladium*.

The 605 figures in the book vary widely in illustrative value. Some of the drawings suggest uncertainty on the part of the artist regarding details of form and structure which are critical in identification of a fungus or in determination of its life history. Some obviously represent artefacts of shrinkage or of abnormal growth. Figs. 24-31, e.g., are not typical of *Coccidioides immitis*, are therefore misleading, and should have been omitted. There are several repetitious figures. The cells shown in Fig. 63, interpretation of which is equivocal, appear in three other figures. Some of the color plates inadequately represent the fungi, are not labeled in a uniform manner, are inadequately labeled or bear reversed labels. For example, the structures in Table II, center, d, represent either incorrect interpretation and depiction of the structures present or are not *Histoplasma*, as labeled. Table VIII, F appears to represent *Coccidioides* (?) rather than *Actinomyces*. Table XV, D is difficult to interpret but apparently the labels for it and for XV, A are reversed. Clinical pictures are for the most part excellent to adequate, but poor reproduction mars a few.

The student without a sound background in mycology and acquaintance with the early papers in medical mycology may find the reviews of taxonomic opinions and nomenclatural changes and the schema representing theoretical life histories and relationships confusing. The professional mycologist will find this compendium of historical viewpoints useful and Prof. CIFERRI's concepts stimulating. The author's thorough knowledge of mycology and of the literature of medical mycology, his challenging viewpoints and his long experience in the field will insure the acceptance of this and its companion volume as indispensible additions to a complete working library.

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CUMMINS, GEORGE B. Illustrated Genera of Rust Fungi. Burgess Publishing Co., Minneapolis, Minn., 1959 pp. ii, 131. $4.50.

This is an excellent manual for beginning students as well as for mycologists, plant pathologists and other scientists not specialists in the study of rust fungi. There are many illustrations, including 9 figures in the introductory section and un-numbered figures for each of the treated genera. These figures include photomicrographs, schematic sketches and camera lucida drawings. In addition, ample literature is cited to permit the investigator to locate further reference material for identification to the species level. Included in the bibliography are 17 descriptive manuals and 37 regional lists of the rust fungi of the world. A twelve page introductory discussion includes such topics as spore forms, life cycles, host plant-rust fungus associations and taxonomic problems of rust fungi. Then follows a key to the 95 genera which are discussed in the manual. Printing errors are few in number.

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Staining Procedures Used by the Biological Stain Commission, 2nd Edition, Revised by H. J. Coon, Geneva, N.Y., M. A. Darrow and V. M. Emmel, Rochester, N.Y.: The Williams & Wilkins Co., Baltimore, 1960. 289 pp., $ 5.00

Publication of this book has been long awaited. It makes available in one volume material which had been published intermittently in looseleaf form from 1943 to 1955. The information presented has been completely revised by the authors and a group of specialists.

The introductory chapter deals with general methods for fixation, dehydration, embedding, and related techniques. Specific subject matter has been organized into 3 sections:

Part I. Animal Histology. The six chapters in this section deal with methods for general tissue, connective tissue, neurological tissues, blood, bone and bone marrow and cell constituents.

Part II. Plant Histology. This section with 3 chapters, covers methods for general tissue, pollen and pollen tubes and cytology.

Part III. Microbiology. The final two chapters are concerned with stains for microorganisms in smears and in sections.

An extensive bibliography and index complete the contents of the book.

The objective of the authors to present in a standardized form the staining procedures utilized by the members of the Biological Stain Commission has been accomplished in a precise and practical manner. This compendium of formulae and techniques will prove valuable to all engaged in histological and cytological work.

Medical mycologists will be disappointed in the failure to present the stains that facilitate the detection and identification of pathogenic fungi in animal tissues. In future editions, it would be desirable to include the Gridley, periodic acid-Schiff, mucicarmine and methenamine silver nitrate procedures in the chapter that describes stains for microorganisms in sections.

The growth of interest in and the utilization of fluorescent-antibody staining procedures for the detection and identification of microorganisms in tissues should also be recognized. The Biological

Stain Commission could establish suitable specifications for the fluorochromes used in these technics.

This book is printed with highly legible type and has a sturdy, plastic spiral binding. The card-board cover, however, will not withstand hard usage.

LIBERO AJELLO

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M. B. ELLIS, Commonwealth Mycological Institute, Kew, England: DEMATIACEOUS HYPHOMYCETES: I. Mycological Papers No. 76, Commonwealth Mycological Institute, Kew. 27 April 1960. 36 pp. 10s.

This contribution represents a continuation of the series of reports by M. B. ELLIS in which various genera of fungi are redefined and numerous species are reassigned. The work is done on the basis of careful culture studies in which conidia, conidiophores and related structures are interpreted in the light of recent information, especially in relation to spore production.

In this report *Acarocybe* SYDOW is redefined, the type species is redescribed and a new species added; the new genus *Acarocybella* is described, based on one *Cercospora*-like species; the genus *Pithomyces* BERK. & BR. is brought up to date to replace *Neomichelia* PENZIG & SACCARDO and *Scheleobrachea* HUGHES; and 10 species are added to *Corynespora* GÜSSOW redescribed by M. B. ELLIS in 1957.

In addition to 2 species described as new in *Pithomyces*, 7 transfers were made of species which had previously been assigned to such genera as *Helminthosporium*, *Clasterosporium*, *Sporidesmium*, *Dicoccum*, *Piricauda* and *Scheleobrachea*. *P. flavus* BERK. & Br. is listed as the type. In addition to 6 new species described in *Corynespora*, transfers were made of species which had been included previously in *Mystrosporium*, *Sporidesmium*, *Clasterosporium*, *Coryneum*, and *Helminthosporium*. It is of interest to note that *Corynespora olivacea* now includes some of the material which had previously been assigned to *Exosporium tiliae* in North America and Europe.

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A. Johnston, Dept. of Agriculture, Federation of Malaya: A Supplement to a Host List of Plant Diseases in Malaya. Mycological Papers No. 77, Commonwealth Mycological Institute, Kew. 25 May 1960. 30 pp. 7 s. 6 d.

A list of 385 species of fungi parasitic on 318 species of vascular plants, 15 species of fungi, and 8 species of insects in Malaya.

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AMERODISCOSIELLA, A NEW GENUS OF THE LEPTOSTROMATACEAE FROM CAMBODIA

by

Marie L. Farr

(Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Md.)

(with 3 figs.)

(16.IX.1960)

Among some dried specimens received from Cambodia was one consisting of two small plants of Bermuda Grass *Cynodon dactylon* (L). Pers. possibly killed by a virus. Certain non-parasitic fungi (such as *Dinemasporium gramineum* Lév. and *Dimeriella erysipheoides* (Ell. & Ev.) Farr) were present in small quantities and one of these was apparently undescribed. This interesting fungus was characterized by minute, black, flat, superficial fruiting bodies resembling those of the smaller, astomous members of the Microthyriaceae or Micropeltaceae. Microscopic examination, however, revealed an imperfect fungus with relatively large, reniform spores with a long, distinct cilium at each end. No free mycelium was evident. Because the fruiting bodies were widely scattered and very few in number they were not sectioned, but from the crushed mature pycnidia it seemed likely that conidiophores were lacking, early evanescent, or poorly developed.

In the effort to fit this clearly distinct fungus into a known genus a thorough search of the literature dealing with Leptostromataceae having ciliate conidia was carried out, but none of these fungi were found to possess a combination of radiate, scutate fruit bodies and reniform, ciliate, one-celled pycnidiospores.

Tracyella, a small leptostromataceous genus with one of its species on grass, is characterized by conidia with one filamentous appendage at each end, but is entirely different in habit and appearance. Five species have been assigned to *Tracyella*, but according to Petrak's (1947) discussion the genus appears to be a somewhat heterogeneous mixture of fungi of varying structure; hence descriptions, illustrations, and specimens (where available) of each species were examined. The new fungus immediately appeared unquestionably distinct from all of these except *T. aristata* (Cke.) Tassi by its scattered, minute, orbicular pycnidia. The unusual shape of its spores set it apart further. Those of *Tracyella spartinae*

(Peck) Tassi and *T. minima* (Berk & Curt.) Moesz, of which material was available for examination, are narrow and more or less cylindrical and borne on readily discernible conidiophores. Those of *T. androsovszkyi* Moesz likewise are developed on a layer of conidiophores, some of which, in turn, are inserted on a columella according to Moesz's (1914) descriptions and illustrations. They have a long cilium on the upper end, a short arista on the lower, and are described as elliptical-fusoid, with some appearing ovate and obovate in the illustration. *T. aristata* (Cke.) Tassi, judging from Cooke's original description of his „*Leptothyrium aristatum*" possesses pycnidia similar in structure and habit to the Cambodian fungus, but has cylindrical conidia. Type material of that species, if available for study, might possibly show it to be more closely related to, or even congeneric with, the fungus from Cambodia rather than with the other species of *Tracyella*. Certainly the truly ciliate spores exclude Cooke's fungus from *Leptothyrium*.

The monotypic genus *Chaetalysis* Peyr. is of more robust appearance and differs in its habitat on wood and bark; furthermore it has conidiophores and catenate, oblong to cylindrical conidia ciliate only on the distal end.

In other leptostromataceous genera with radiate fruit bodies and similarly appendaged conidia the latter are septate. The small genus *Discosiella* Syd. is perhaps the closest relative of the fungus at hand, differing mainly in possessing two-celled conidia. The pycnidia are described as dimidiate and subsuperficial. The conidia of *D. cylindrospora* Syd., the type species, are straigth and cylindrical with obtuse ends while those of *D. acrocomia- maculiformis* Bat. are straight and fusoid and those of *D. vochysiae* Bat. & Lima cylindric-fusoid; those of *D. longiciliata* Agnihothrudu are "subcylindrical with obtuse ends, slightly narrower at the base often allantoid or botuliform, rarely straight". Judging from descriptions and illustrations of these four species, there appears to be considerable varation among them, in addition to certain discrepancies or differences of interpretation. The cilia of *D. vochysiae* measure 1.5—7.5μ those of *D. longiciliata* are 16—28μ (often exceeding the conidial length); those of the former species are described as one-septate, but pictured as continuous and guttulate. Cilial length in the other two species is of intermediate range. The generic description makes no mention of cell arrangement in the pycnidial walls, but that of *D. cylindrospora* is described, and that of *D. longiciliata* illustrated, as radiate. In *D. acrocomia-maculiformis* the wall cells are stated to be poorly defined, whereas in *D. vochysiae* they are depicted as polygonal and the illustration indicates no trace of a radial arrangement.

Leaf spot formation is not mentioned as a generic feature, but described for all species except *D. vochysiae*. This character is considered sufficiently important to separate other genera of the Sphaeropsidales, as for example *Rhabdospora* from *Septoria*.

No mycelium is mentioned in the generic diagnosis, but SYDOW (1912) states "this new genus (Discosiella) is related to *Discosia* but differs in the bicellular spores and by the presence of a subiculum." Free mycelium is attributed to *D. cylindrospora* and the pycnidia of *D. vochysiae* are described as seated on a thin subiculum, while all reference to mycelium is omitted for *D. acrocomia-maculiformis* and ,,superficial mycelium (is) totally absent" in the case of *D. longiciliata*. The lack of spot formation by *D. vochysiae* and a comparison of the illustrations of *D. vochysiae* and *D. longiciliata* cast some doubt on the closeness of the relationshop of these two species.

In general the fungus under study has in common with *Discosiella* (at least in part) its habitat on old leaves; its superficial, orbiculate, scutate, radiate, astomous pycnidia; its lack of (or poorly developed?) conidiophores; and its unpigmented conidia with one filiform appendage at each end. It differs from that genus in its consistently one-celled reniform conidia; the lack of any evident free

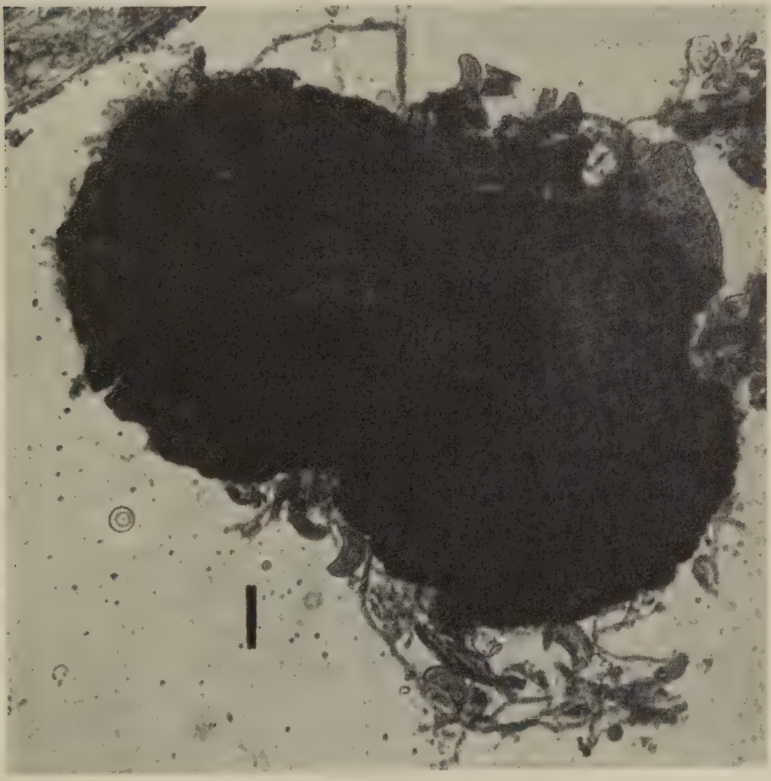

Fig. 1. *Amerodiscosiella renispora*. Pycnidia and conidia, approx. × 500.

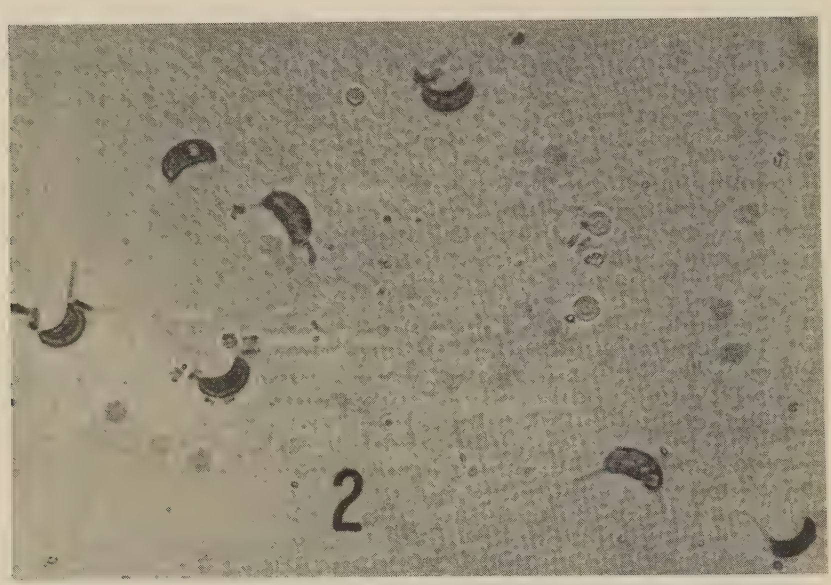

Fig. 2. Conidia, approx. × 500.

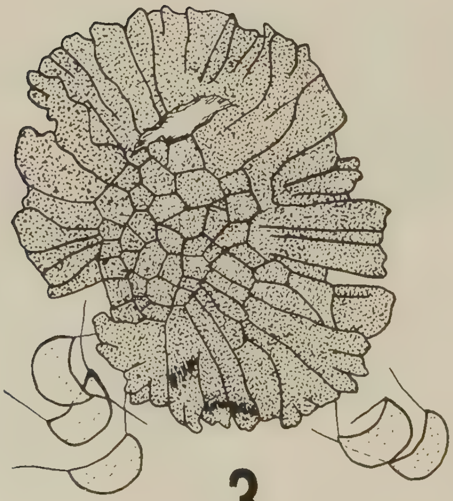

Fig. 3. Camera lucida drawing of small pycnidium and conidia, c. × 870.

mycelium; and the absence of leaf spot development. Of the *Discosiella* species it seems to approach most closely *D. longiciliata* in shape of conidia and appearance of pycnidia. No thickened walls, such as are shown in AGNIHOTHRUDU'S (1958) figure, have been observed in this fungus.

Amerodiscosiella gen. nov. (Sphaeropsidales: Leptostromataceae).

Mycelium librum nullum; pycnidia superficialia, scutata, radiata, orbicularia vel suborbicularia, membranacea, astoma; conidiophora non visa; conidia reniformia, subhyalina vel pallide luteola, unicellulata, utrimque uniciliata. TYPUS: *A. renispora* sp. nov.

Free mycelium none; pycnidia superficial, scutate, radiate, orbicular or suborbicular, membranous, resembling astomous microthyriaceous fruit bodies; conidiophores not observed; conidia reniform, subhyaline or pale yellowish, one-celled, with a cilium at each end. TYPE SPECIES: *A renispora* sp.nov.

A. renispora sp. nov. (figs. 1—3).

Pycnidia 80—110μ diam., specie irregulariter dehiscentia pariete e cellulis polygonalibus ad centrum et cellulis elongatis rectangularibus ad peripheriem composita; conidia contextu irregulariter granulatu, reniformia extremiorum obtusiorum, 11—15 ×5—7μ; cilia 5—13μ longa. Ad foliis *Cynodontis dactylonis*.

Pycnidia 80—110μ diam.; wall made up in the central part of irregularly rectangular or polygonal cells 5—8μ diam., replaced in the peripheral region by radiately arranged elongated cells to 25μ long and 4—8μ wide, usually cleft or bilobed at the margin; basal membrane subhyaline to pale brownish; dehiscence apparently irregular; conidia subhyaline to pale yellow, of irregularly granular context, reniform with obtuse ends, 11—15 × 5—7μ; cilia 5—13μ long. TYPE: On leaves of *Cynodon dactylon* (L.) PERS., Phnom Penh, Cambodia, LITZENBERGER 27, 1959.

I would like to express my appreciation to Mr. JOHN A.STEVENSON and Dr. CHESTER R. BENJAMIN for critically reading the manuscript and to Miss EDITH K. CASH for correcting the Latin diagnoses.

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2 CASOS POCO COMUNES DE ASPERGILOSIS PULMONAR Y PLEURAL. ¹)

por

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(10 figs.)

(14.IX.1960)

La casuística acerca del tema abunda; sobre todo en los últimos años con el incremento de las intervenciones de la cirugía toráxica se han observado numerosos casos de micetomas pulmonares y entre ellos cierto número de „aspergilomas".

En ésta misma revista se han publicado muchos reportes acerca de casos de ésta naturaleza.

El propósito de éstas lineas no es aumentar el número de la casuística, dar un resúmen bibliográfico de todas las observaciones hasta ahora hechas o entrar en pormenores acerca del género *Aspergillus*, el cual fué descrito por Micheli²) desde hace más de dos siglos, sino más bien hacer conocer brevemente unos hallazgos raros que caracterizaron nuestros dos casos.

El primero, del año 1957, era un aspergiloma típico secundario (Monod (1951), Vellios (1957), Segretain & Vieu (1957), Enjalbert y col. (1957) y otros) en el cual sí pudo comprobarse sin lugar a dudas el mecanismo primitivo que ha preparado el terreno para la formación secundaria del micetoma. Además, la sintomatología en ésta paciente y algunos hallazgos micológicos nos parecen excepcionales.

La segunda observación del año 1954, caso autópsico, presentó una diseminación pleural del hongo con foces múltiples, forma de micosis de la cual no hemos encontrado descripción alguna en la literatura al respecto.

Es de notar además que casos de aspergilosis pulmonar en Venezuela, aparentemente no són frecuentes.

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¹) Trabajo presentado en forma abreviada en el II Congreso Latinoamericano de Anatomía Patológica, São Paulo, Brasil, 1958 y en el IV Congreso Venezolano de Tisiología y Neumonología 1959, Valencia, Venezuela.

²) Pier Antonio Micheli. — Nova Plantarum Genera Iuxta Tourneforti Methodum Disposita. Typis Bernardi Paperini, Floreciae, 1729, pagg. 212—213.

Fuera de nuestros casos sabemos unicamente de observaciones hasta ahora no publicadas de ANGULO ORTEGA (1958), las cuales representan aspergilosis pulmonares secundarias y difusas parecidas en sus manifestaciones anatómicas a los conocidos casos de VIRCHOW (1885).

PRIMER CASO. see p [illegible]

Datos clinicos: Señora (No S.V. 738—1957) de 28 años de edad, casada, con 7 hijos, residenciada en el caserío Loma del Carmen, cerca de Jají, Edo. Mérida, de ocupaciones domésticas y que vive en una casa de campo.

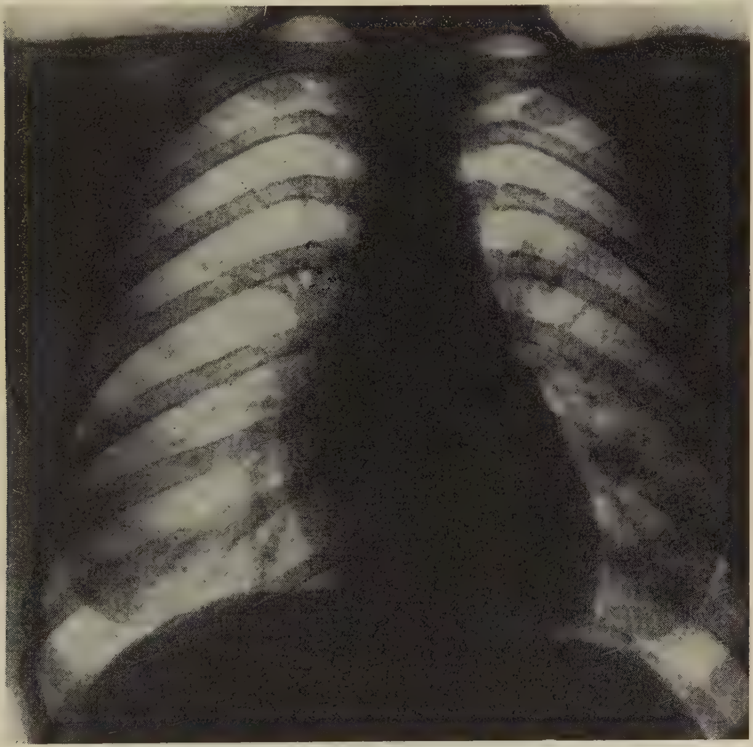

Fig. 1. Radiografía de ingreso. Imagen poco densa no bien delimitada en región paracardíaca izquierda, a nivel del 8° arco costal posterior.

La paciente ocurrió en el mes de julio de 1957 al Dispensario Antituberculoso de Mérida con el fín de obtener un certificado de salud. La imágen fluorofotográfica (fig. 1) reveló una pequeña sombra densa, paracardíaca, izquierda, motivo por el cual la paciente fué citada a la consulta.

En el curso del interrogatorio, la enferma relató que a los 10 años de edad (hace 18 años) había tomado un dia, en compañía de sus amigas, un poco de ,,guarapo fuerte'' y que había presentado enseguida una hemoptisis de regular importancia, durante un ataque de risa. Las hemoptisis se repitieron desde entonces con regular frecuencia y con intervalos de 3 a 4 meses hasta 1 año, sin ninguna relación aparente con las menstruaciones y hasta la fecha del exámen, sin ningún otro motivo aparente, al no ser el de acostarse sobre el lado izquierdo y boca arriba, lo cual era suficiente en la mayoría de los casos para producir un acceso de tos seguido de una hemoptisis. La paciente refiere haber sufrido durante su último embarazo de una paralisis unilateral total de 3 dias de duración. Además, hace pocos meses, tuvo un proceso neumónico del lado izquierdo, acompañado de fiebre alta, dolor del pecho y expectoraciones muco-purulentas y hemoptóicas. El proceso neumónico desapareció aparentemente a los pocos dias, con un tratamiento a base de antibióticos, cuya clase y dosis no fué especificado. No fué posible obtener datos complementarios del médico tratante.

En los últimos meses, la paciente observó un aumento de intensidad y número de las hemoptisis, las cuales empezaron causándole una debilidad general, mareos frecuentes, trastornos del equilibrio, nauseas y dolor occipital. Se sentía obligada a permanecer dias en la cama. Para el momente del exámen, la paciente no acusó ninguna sintomatología respiratoria, fuera de lo arriba expuesto.

Fig. 2. Imagen tomográfica de la lesión en posición acostada.

Al exámen clínico se trataba de una paciente en condiciones generales regulares, con un peso corporal de 45 kilos para una talla de 1,53 mts. muy pálida con mucosas anémicas, taquicardia, de 90 pulsaciones per minuto y con una T.A. de 110/70. Fuera del hallazgo radiológico de un pequeño infiltrado, del tamaño de una cereza, en región paracardíaca izquierda, no se encontró ningún otro dato patológico. En vista de los sintomas acusados por la paciente, que relacionaba la aparición de las hemorragias pulmonares con la posición corporal, se le indicó acostarse boca arriba sobre la camilla; a los pocos segundos de estar acostada, la paciente presentó un acceso de tos, acompañado de 4 o 5 esputos hemoptóicos, de una cantidad de 20 cc. de sangre. Luego se levantó sin que se hubieran repetido las expectoraciones hemoptóicas.

Dada la imposibilidad de una hospitalización inmediata se sometió durante un mes a un tratamiento antianémico ambulatorio, a base de Ferronicum. El dia 23-8-57 ingresa al Sanatorio ,,Venezuela'', bajo el diagnóstico provisional de ,,pulmón sangrante''. El estado general había mejorado considerablemente, la paciente era ahora asintomática y no habiá presentado más esputos hemoptóicos.

Repetidos exámenes para el bacilo de Koch resultaron negativos, hasta en contenido gástrico cultivado. La sedimentación globular era de 10/30. El exámen de sangre reveló una discreta anemia con 4.000.000 de globulos rojos y 12,5% de hemoglobina (van Slyke). El cuadro hemático diferencial, con una cifra total de 8.000 globulos blancos, llamó la atención por una eosinofilia de 27 % y una linfocitosis de 31 %; neutrófilos segmentados 42. El exámen de heces reveló una ascaridiasis.

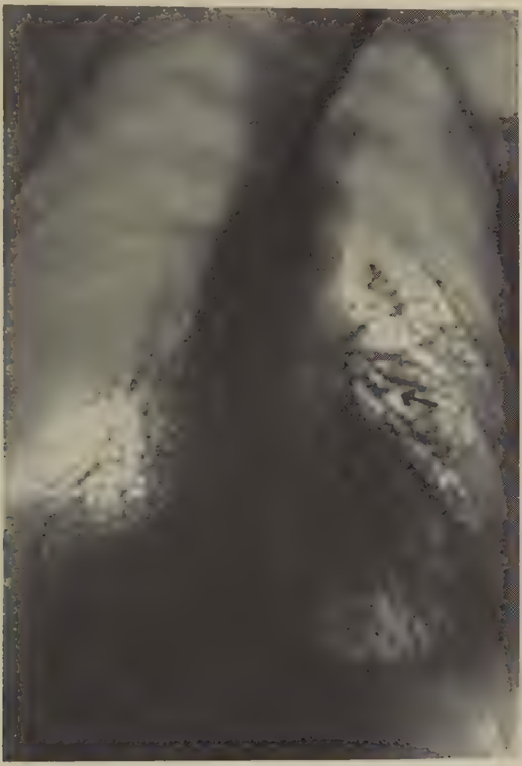

Fig. 3. Broncografía. Amputación de un bronquio segmentario apical del lóbulo inferior izquierdo.

Las imágenes radiológicas no revelaron ningún cambio en comparación con los primeros exámenes (fig. 1). El plano tomográfico de 6 cms. (fig. 2) demostró la presencia de una sombra densa paracardíaca izquierda, de limites bien definidos y del tamaño de una ciruela, con una zona de aclaramiento de limites irregulares.

La evolución del proceso durante 18 años, nos dejó pensar en la presencia de una bronquiectasia sacciforme, circunscrita y aislada, una endometriosis, aneurísma pulmonar y en un tumor pulmonar benigno, eventualmente hemangioma. Pudimos excluir con cierta seguridad un proceso tuberculoso; no pensamos en micosis. La endometriosis podría eliminarse por no existir ninguna relación aparente entre los períodos de gestación y las hemorragias pulmonares.

El exámen funcional, excluyó la presencia de un corto circuito circulatorio intrapulmonar. Al exámen radioscópico no existía ninguna pulsación de la imágen sospechosa, ni tampoco cambiaba de forma o tamaño durante el Valsalva.

La angiografía selectiva del pulmón dió el siguiente resultado: maracada desviación de la arteria apical lateral y de la arteria principal del lóbulo inferior, formando estas dos un angulo abierto. Disminución de la luz arterial, retardo circulatorio, con retención del medio de contraste en la región sospechosa. No hay signos de un aneurisma arteriovenoso; los hallazgos hablan más bien en favor de un desplazamiento vascular, por una formación tumoral con retardo circulatorio en la región correspondiente. De ésta manera há podido excluirse también, la presencia de un aneurisma arteriovenoso.

Finalmente, la broncografía (fig. 3) demostró un ,,stop'' con amputación bronquial, a nivel de los segmentos apicales del lóbulo inferior izquierdo.

Sin llegar a una conclusión diagnóstica definitiva se decidió practicar una intervención quirúrgica, previo diagnóstico de ,,tumor pulmonar sangrante, del lóbulo inferior izquierdo''. El 31-10-57 se efectuó después de un tratamiento antianémico complementario, la resección del lóbulo pulmonar inferior izquierdo (anestesia Dr. E. Briese).

La paciente toleró bien el acto operatorio; el post-operatorio fué normal y la enferma fué dada de alta por curación, al mes y medio de haber sido operada, en buenas condiciones generales.

Un exámen de control de fecha 11-6-58,seis meses y medio después del acto quirúrgico, reveló una re-expansion pulmonar completa; la paciente está sin sintomatología alguna.

Examen Anatomopatológico (E No 5.824).

Enviaron un lóbulo pulmonar inferior y dos piezas, una de 24 y otra de 6 mms. de largo.

El lóbulo pulmonar insuflado, no fijado, con dos ramas bronquiales ligadas a nivel del hilio, demuestra en ésta región una retracción en la pleura visceral con adherencias pleurales y una zona pulmonar correspondiente circunscrita, de color morado-violáceo y de consistencia aumentada (atelectasia parcial).

Disecando la rama bronquial superior, se encuentra, a unos 2 cms. de la ligadura, una dilatación circumscrita del bronquio de 2 cms. de diámetro, que contiene masas semi-sólidas, grumosas de color grisáceo-negrúzco, que no pueden extraerse ,,in toto'', sino que se parten en varios pedazos al sacarlas y que estaban adheridas a la pared de la cavidad. La superficie de la cavidad demuestra un color rojo negrúzco. En la vecindad de la misma, se nota en el parénquima pulmonar, un tejido cicatricial con antracosis. Al sondear la circunferencia de dicha cavidad, se observan varios bronquios, que desembocan hacia la misma y los cuales contienen una substancia espesa, de aspecto de mucosidades y un liquido puriforme. Uno de los bronquios está en comunicación con la rama bronquial inferior ligada, a nivel del hilio. En otro de los bronquios pequeños, cerca de la cavidad, se encuentra enclavado un cuerpo duro, de contornos irregulares, de color amarillento y de 5 mms. de largo que dá la impresión que se tratase de una particula ósea, de un grano de arena o de una pequeña piedra.

Se interrumpe la disección de la pieza fresca en este momento para fijarla y tomar fotografias (fig. 4) de la pieza pulmonar y (fig. 5) del contenido de la cavidad y del pequeño cuerpo extraño del bronquio.

Macroscópicamente, teniamos la impresión que se trataba de una bronquiectasia circunscrita, con formación de una cavidad, de origen no determinado o debida a la aspiración de un cuerpo extraño, que se había enclavado. El contenido de la cavidad, interpretabamos eventualmente, como masas necróticas, mezcaldas con sangre antigua — de las hemoptisis relatadas clinicamente —. Pensabamos además, en la posibilidad que se tratara eventualmente de un foco ganglionar

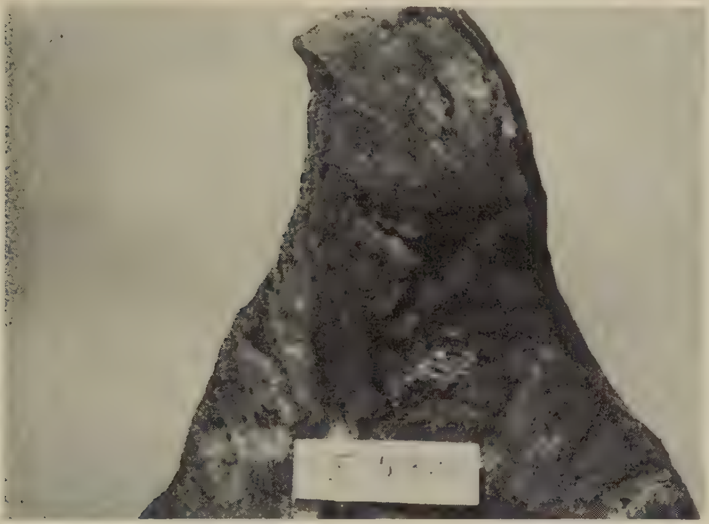

Fig. 4. E. 5824. Pieza operatoria (lóbulo pulmonar inf. izqu.) fijada, vista del hilio con cavidad vacia en comunicacion con 2 bronquios segmentarios. Contenido de la cavidad, extraido.

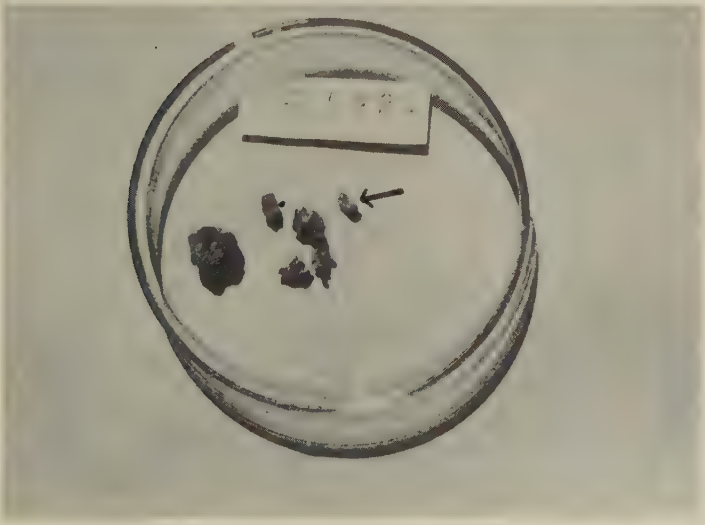

Fig. 5. Contenido extraido de la cavidad en la figura 4, fragmentado durante la extracción. La flecha indica el grano de arena.

de un antiguo complejo tuberculoso primario, perforado hacia la luz del bronquio. Pudimos excluir macroscópicamente, tumor o anomalias vasculares.

La disección posterior de la pieza pulmonar fijada, mediante discos finos de 2 a 3 mms. de grosor, revelaba sólo bronquios discretamiente dilatados en forma difusa, en la periferia, conteniendo mucosidades.

Fueron exáminados microscópicamente mediante numerosos cortes:

1. La rama bronquial superior, ligada a nivel del hilio.
2. La pared de la cavidad descrita (se incluyeron 5 piezas con los bronquios que se ramificaron de la misma).
3. El contenido grumoso de la cavidad.
4. El cuerpo extraño duro del bronquio, cuya mitad fué conservada para exámenes químicos.
5. Varias piezas del resto del lóbulo pulmonar.
6. Las dos piezas de 24 y 6 mms. de diámetro respectivamente, que se enviaron junto con el lóbulo pulmonar y que correspondian a pequeñas tumoraciones a nivel del hilio.

1. Encontramos una marcada bronquitis y peribronquitis crónica inespecífica, con numerosas células eosinofílicas.

2. La superficie interna de la cavidad, hacia la cual desembocan varios bronquios, no demuestra en ningún sitio, tejido epitelial. Tampoco se observan glandulas mucosas, tejido cartilaginoso o tejido muscular. La superficie está constituída por tejido de granulación inespecífico, rico en capilares sanguíneos, con densas infiltraciones linfoplasmocitarias y un número variable de eosinófilos. Hacia afuera, se nota una capa gruesa de tejido fibroso, con marcados procesos de vasculitis crónica obliterante y depósitos de pigmento de antracosis.

En el tejido pulmonar vecino aireado, se observan, en un sitio circunscrito, acumulos de hifas y esporas, Atravesando la capa del tejido de granulación y desembocando hacia la luz de la cavidad, se observan en varios sitios, claramente, vasos sanguíneos, (arteriolas y venolas) con paredes esclerotizadas.

Adherido al tejido de granulación, se ven discretas cantidades de masas de detritus tisular y celular con granulocitos destruidos. En la luz de los bronquios, cuyas paredes estan densamente infiltradas por linfo, plasmo y granulocitos, se encuentra pus y mucosidades.

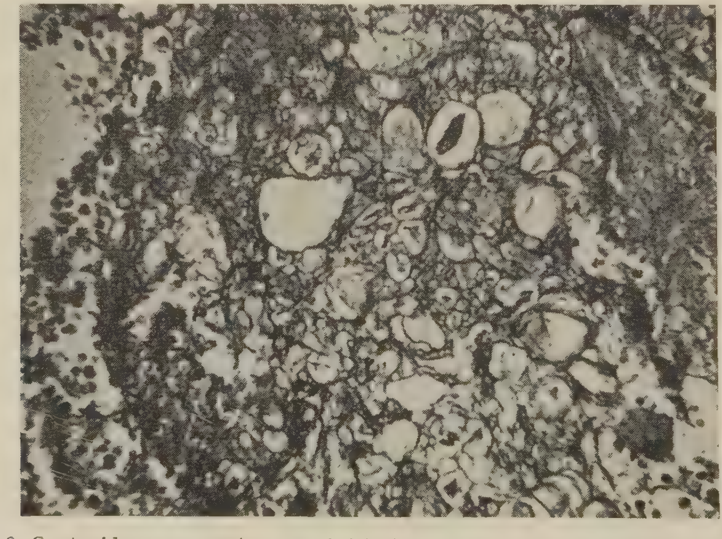

Fig. 6. Contenido grumoso (grano micótico). Colorac. Hematox.—Féric. Copia de una transparencia en color. Zeiss Opton Ocul. 8—1, 6; Obj. 16.
Hifas micelares hinchadas y vacuolizadas (alteraciones regresivas secundarias).

3. El contenido grumoso de la cavidad, incluido en parafina se dejó cortar fácilmente y demostró en Hematoxilina-Eosina las estructuras de un seudo — tejido o hifénquima. Se reconocieron formas celulares de 2—3 micras de diámetro, redondas, ovaladas o alargadas; en las últimas se observaron tabiques y ramificaciones. Además se vieron masas amorfas de aspecto de tejido necrótico, granulocitos, eritrocitos, depósitos de pigmento de hemosiderina y de sales de cal. En unos campos se observaron imágenes de dificil interpretación, como acumulos de vacuolas de diferentes tamaños y de contornos irregulares y corpusculos redondos sin núcleos celulares definidos. Las imágenes en cuestión, nos parecieron en el primer momento, correspondientes eventualmente a tejido adiposo necrótico (fig. 6) diagnóstico que tuvimos que corregir posteriormente. Se trata más bien de filamentos micelares vesiculados (alteraciones regresivas) como lo ilustran SÉGRETAIN & VIEU (1957) en su figura 5.

Los bordes de la masa parecian anfractusos e irregulares. Cerca de los bordes, pero también en el interior de la masa, se encontraron abundantemente fructificaciones típicas de la forma de reproducción del género *Aspergillus*, es decir, hifas conidióforas alargadas, ensanchadas en su parte distal en forma de clava o vesicula con presencia de una corona simple de esterigmas, por encima de la vesicula (fig. 7).

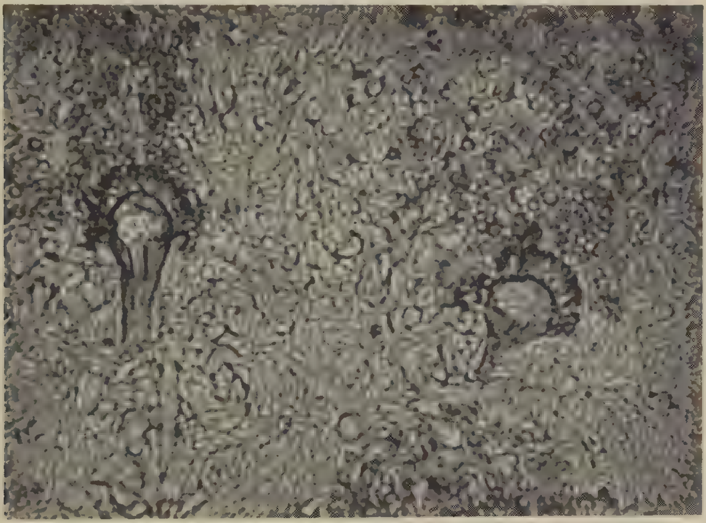

Fig. 7. Contenido grumoso. (grano micótico). Colorac. Fibrina (Weigert). Copia de una transparencia en color. Zeiss Opton Ocul. 8; Obj. 63.
Cabezuelas fructíferas (conidióforas con esterigmas) y conidios de color amarillento propio.

En un campo (fig. 8) se observa una hifa conidiófora cortada en dirección longitudinal, cuya longitud es de unas 80 micras. El ancho de la vesicula es de 15 micras. La longitud de los esterigmas 6—8 micras y su ancho de 1—1,5 micras. En esta y otras fructificaciones cortadas en dirección longitudinal, se reconoce que los esterigmas no estan dispuestos cubriendo de manera completa toda la vesícula, sino colocados en los dos tercios de su parte distal y orientados más o menos paralelos entre sí y en dirección del eje longitudinal de la formación fructífera.

En otros campos se observan acúmulos de células esféricas o ligeramente ovaladas, de membrana lisa y de 2,5—3 micras de diámetro. Estas formaciones corresponden a conidios desprendidos de las hifas conidióforas.

Las fructificaciones y los conidios desprendidos demuestran en los cortes un color propio entre amarillento y marrón claro y dieron la imágen mas contrastada en la coloración de Fibrina (Weigert); no se tiñeron can PAS de color rojo.

4. La mitad del cuerpo extraño duro, la sometimos a una discreta decalcificación. En los cortes microscópicos, se reconocieron masas amorfas no homogéneas. No pudimos ver ni estructuras de tejidos o dé células. Sobre todo no era posible observar imágenes de un tejido óseo. Tampoco se visualizaron elementos micóticos o cristales con estructuras definidas.

5. En los numerosos cortes examinados del resto del lóbulo pulmonar, que fueron practicados de varios sitios se observaba bronquios discretamente dilatados con inflamación mucopurulenta de grado e intensidad variable, en distintas zonas. No se encontraron eosinofilia ní tampoco elementos micóticos.

6. Las dos piezas del hilio pulmonar, resecadas independientemente del lóbulo pulmonar, representan ganglios linfáticos grandes con antracosis.

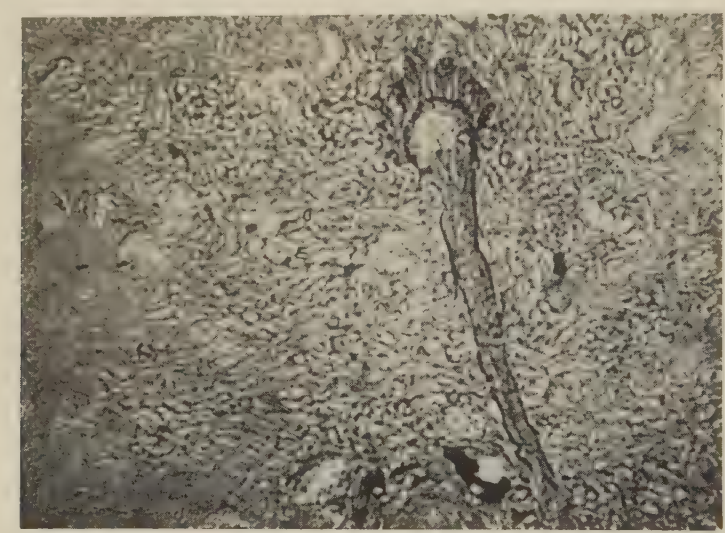

Fig. 8. Contenido grumoso (grano micótico). Colorac. Hematox. — Féric. Copia de una transparencia en color. Zeiss Opton Ocul. 8; Obj. 63.
Hifa micelar con cabezuela fructífera. La disposición y dirección de los esterigmas indíca que se trata de elementos de *Aspergillus fumigatus*.

Examen Químico.¹)

El resultado del exámen químico del cuerpo extraño intra-bronquial (No 4), es el siguiente:

La pieza pesa 27,8 mgrs y contiene 7,6 mgrs de ácido silícico ($Si\ O_2$) es decir 27,3 %.

Además se encontró CO_2, Mg y Ca. No se pudo comprobar fósforo o substancias orgánicas.

Se trata pues, de materia anorgánica (grano de arena o pequeña piedra). — Se excluyó tejido óseo.

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¹) El exámen fué realizado por el Prof. Dr. Carl Seelkopf, Director del Instituto de Investigaciones Químicas de la Facultad de Farmacía de la Universidad de Los Andes. Aprovechamos la oportunidad para darle nuestras más sinceras gracias al Dr. Seelkopf, quién nos há honrado ya repetidas veces con su valiosa colaboración.

COMENTARIOS

Las hemoptisis repetidas durante muchos años, el aspecto radiológico y las manifestaciones anatómicas en nuestro caso eran más bien típicos para un -,,aspergiloma''; no extrañaba tampoco la negatividad del esputo en cuanto a la presencia de elementos funginos. Sin embargo, nos parece excepcional, el hecho de que se producía en éste caso de ,,pulmón sangrante'' una anemia que exigía tratamiento y la cual fué curada finalmente mediante una resección pulmonar parcial. Además, llamaba la atención en el caso descrito, que las hemoptisis se producian unicamente en ciertas posiciones, hecho que encuentra su explicación por movimientos del grano dentro de la cavidad el cual taponaba la salida o destapaba la misma según los cambios de la posición puesto que aparentemente estaba solamente parcialmente adherido.

La presencia de abundantes fructificaciones permitó la determinación sistemática del hongo solamente a partir del exámen morfológico como *Aspergillus fumigatus* FRES. 1863. Si bien éste hallazgo según CIFERRI (1960) es raro, fueron descritos casos con fructificaciones a nivel de cavidades bronquiales y pulmonares por ENJALBERT y col., (1957) PARINI y col. (1957) y otros. Su presencia indica que entre el grano y la pared de la cavidad existía un espacio libre y que la cavidad estaba en comunicación con el trayecto bronquial permitiendo de esta manera la llegada de aire hacia el grano.

El hecho de que las fructificaciones se encontraron tambien como sumergidas en el interior del grano debe atribuirse a la acción mecánica del cuchillo del microtomo por el cual fueron transportadas hacia adentro.

No creemos que se hayan desarrollado en el interior del hifénquima como lo describe BOUFFARD (1949) en un micetoma maduromicósico de DJIBUTI.

La gran mayoría de los casos de aspergilosis pulmonar se consideran como secundarios (WAETJEN (1931)) aunque muy a menudo no pueden comprobarse definitivamente las lesiones primarias que en los casos determinados han dado lugar a la infección fúngina secundaria. Entre los casos de aspergilosis pulmonar aparentemente primaria citamos solo los de COOPER (1946) de FINEGOLD y col. (1959) y de OLK. (1958). Nos parece de cierta importancia que en nuestro caso se pudo comprobar un cuerpo anorgánico extraño y aspirado (grano de arena) el cual aparentemente ha producido la lesión primitiva. Suponemos que el cuerpo extraño aspirado se enclavó a nivel de un bronquio conduciendo a una bronquiectasia circunscrita, a pesar de que no se encuentran elementos de una pared bronquial en la pieza de resección.

La supuesta bronquiectasia circunscrita en nuestro caso, pues, se debía con gran probabilidad a la aspiración de un cuerpo extraño; no se trataba como en el caso recientemente publicado por

BRUNNER (1959) de un „verdadero así llamado quiste pulmonar broncógeno", secundariamente contaminado por hongos.

Nuestro caso demuestra entonces como el de BRUNNER (1959) que los hongos mismos aparentemente no conducen a bronquiectasias lo que sostiene MONOD (1951) en sus casos; sino que pueden asentarse en cavidades preformadas o a nivel de un „locus menoris resistentiae" de diferentes clases.

Si bien la cavidad que contenia el grano es antigua, lo que revela la cápsula fibrosa existente, no encontramos en el tejido de granulación que la reviste, células gigantes. Estas, según WAETJEN (1931), pueden contener fragmentos de hongos e indican la cronicidad del proceso micótico. De tal manera que no podemos concluir acerca de la fecha de la contaminación fungina, es decir, si ella existía desde el comienzo de la sintomatología (18 años) o si se produjo en el tiempo subsiguiente.

SEGUNDO CASO

Hombre de 50 años de edad, domiciliado en el pueblo de Chama, murió después de haber estado cierto tiempo enfermo, con un sindrome febril sin que se pudieran obtener datos clínicos precisos. Fué traido al Instituto de Anatomía Patológica, para aclarar la causa de la muerte. — Posteriormente se supo, que fué tratado con antibióticos, sin poder precisar la clase, dosis y duración del tratamiento.

En la autopsia (A.1.129) se encontró (diagnóstico macroscópico abreviado):
Cirrosis hepática atrófica.
Colitis ulcero-hemorrágica, sobre todo en el colon descendente.
Ascaris, tricocéfalos y necatores en el intestino.
Anemia general.
Bronconeumonía rica en edema en ambos lados.

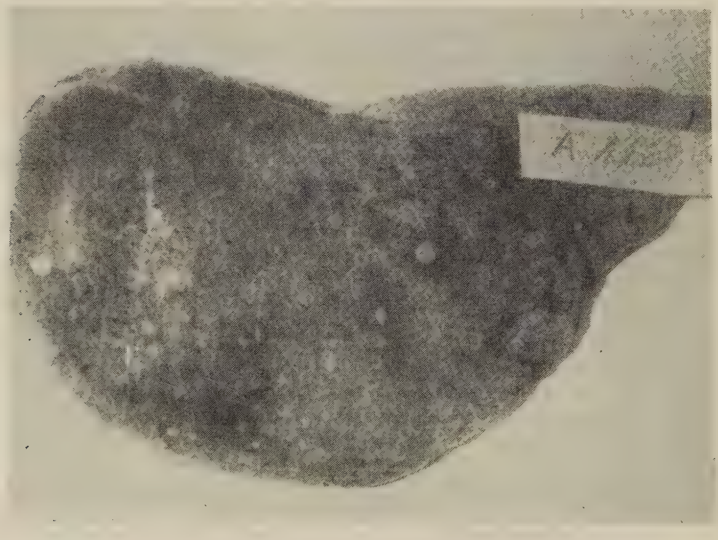

Fig. 9. A. 1129. Focos blanquecinos a veces con halo rojo en la pleura visceral.

Además, en la pleura visceral de ambos lados, una siembre difusa de focos blanquecinos, bien delimitados, discretamente prominentes, de consistencia mediana, de pocos mms. hasta 1 cm. de diámetro con un halo hemorrágico (fig. 9).

Macroscópicamente no fué posible hacer un diagnóstico de estos focos en la pleura. —No se parecian a una siembra típica de focos tuberculosos, ni a metástasis tumorales.

Microscópicamente se encontró:

Pulmones: focos bronconeumónicos pequeños y más extensos en parte hemorrágicos en unos sitios con formación de pequeños abscesos y con marcado edema perifocal. Exclerosis de ramas de la arteria pulmonar y trombosis con reacción inflamatoria a nivel de las paredes vasculares.

A nivel de los focos bronconeumónicos, de los trombes y en los focos de la pleura, se encuentran numerosas hifas micélicas ramificadas y tabicadas, junto con conidios que por sus características morfológicas parecen ser de *Aspergillus* sp. (fig. 10). Los hongos invaden en forma típica paredes bronquiales y vasculares y la pleura (Waetjen (1931)). Se tiñen bien con Hematoxilina Férrica, Fibrina (Weigert) y PAS (según Braun-Falco).

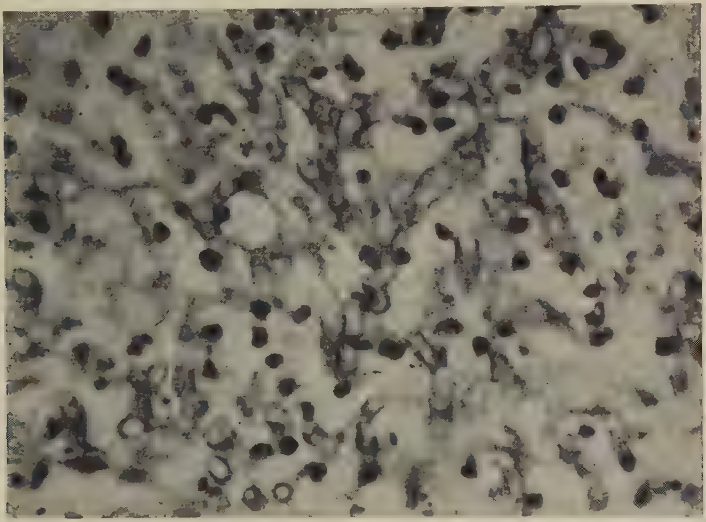

Fig. 10. A, 1129. Microfotografía de un foco pleural. Colorac. Hematox.—Féric. Zeiss Opton Ocul. 8—1, 6; Obj. 16.
Hifas micelares septadas y ramificadas de aspecto de *Aspergillus sp.*

En los demás órganos examinados microscópicamente (higado, colon, riñones, encéfalo, aorta, miocardio, páncreas, glandula suprarrenal y glandula salival submaxilar) se encontró fuera de los hallazgos mencionados en el diagnóstico macroscópico, una esclerosis de las arterias grandes y medianas. No se observan hongos fuera de los pulmones y de la pleura.

COMENTARIOS

En lo que se refiere a la aspergilosis pulmonar y pleural en éste caso, puede tratarse de una micosis primaria, o lo que es más verosímil, de una secundaria, en un sujeto con bronconeumonía banal, que además estuvo en un estado de defensa y resistencia general baja. No pudo determinarse con exactitud, la causa de la invasión tan extensa y masiva por el hongo, aparentemente, poco antes del fallecimiento.

Por otra parte, no se apreciaron lesiones pulmonares antiguas, de tal manera que eventualmente estamos en presencia de una aspergilosis (?) primaria.

Por falta de exámenes micológicos especiales, no tenemos suficiente fundamento para asegurarlo.

Desafortunadamente, en el caso descrito, no se puede concluir tampoco acerca de posibles relaciones patogenéticas, entre el tratamiento de antibióticos y la micosis.

Respecto a éste tema tan de actualidad, nos sea permitido mencionar que en nuestro material autópsico y biópsico, no hemos observado un índice alto de casos de micosis, que eventualmente pueden deberse a un tratamiento excesivo con antibióticos, a pesar de que la administración de estas drogas es muy frecuente en nuestro medio aún sin control médico.

Uno de los pocos casos de aspergilosis pulmonar, que pueden atribuirse eventualmente a una intensa terapia con antibióticos mencionan PARINI, SPROVIERI & SFONDRINI (1957) quienes describen la comprobación de cabezas fructiferas, en el esputo de 3 casos lobectomizados por tuberculosis; los autores discuten sin embargo, en estos casos también una contaminación ocasional durante la intervención quirúrgica.

También relataron unos colegas brasileros, quiénes asistieron a la exposición de nuestros casos en São Paulo, una posible influencia favorable de antibióticos, para el desarrollo de los *Aspergillus* en algunos casos observados.

Quisieramos, sin embargo, hacer hincapié que la revisión de los casos de aspergilosis, de la literatura — a nuestra disposición — no nos há revelado relaciones causales seguras entre el tratamiento de antibióticos y la aspergilosis.

Ilamativo nos pareció y por eso hemos anexado la descripción de este caso a la del anterior, la diseminación o invasion del hongo, tan masiva, en la pleura como hallazgo morfológico no frecuente.

NOTA

En cuanto a la clasificación de la aspergilosis pulmonar en general nos parece acertada la de FINEGOLD y col. (1959) y de NAJI (1959) en primarias y secundarias y la subdivisión de ambos grupos (FINEGOLD y col.) en formas localizadas, invasivas y diseminadas en lugar de la clasificación de ROMINGER & BOEHM (1955) en la cual no se contempló suficientemente la forma localizada (aspergiloma) de la afección.

RESUMEN

Se relatan dos observaciones casuísticas:

En la primera, mujer de 28 años de edad, se encontró en un lóbulo pulmonar resecado una cavidad para-hiliar, en comunicación con los bronquios, que albergaba un aspergiloma, junto con un pequeño grano de arena, cuya naturaleza fué comprobada quimicamente.

La sintomatología pulmonar (hemoptisis repetidas) empezó con un suceso de atragantamiento, al tomar jugo, de ésto hace 18 años. Las hemoptisis se produjeron sólo en cierta posición acostada.

En un segundo caso, autopsia de un hombre de 50 años de edad, casualmente se encontró una bronconeumonía confluente bilateral, con micosis pulmonar (aparentemente aspergilosis) difusa y diseminación del hongo en la pleura.

La siembra focal en la pleura como hallazgo morfológico poco frecuente, motivó la publicación de éste caso.

Al final se hacen breves comentarios sobre la clasificación de la aspergilosis pulmonar.

SUMMARY

The Authors report on two cases of aspergillosis of the lung and of lung and pleura, respectively.

In the first case (a woman, 28 years old) a para-hilar cavity was found in a lobolus of the lung which had been surgically removed. The cavity was connected with the bronchial system and contained a ~~o~~n aspergilloma close to a small sand particle the nature of which was chemically confirmed. Clinical symptoms (repeated hemoptisis) had been present for 18 years after the patient had severely swallowed the wrong way. Occurrence of hemoptoic attacks was dependent on a certain lying position of the patient.

In the second case (autopsy of a man, 50 years old) a confluent bilateral broncho-pneumonia was recorded with a diffuse pulmonary mycosis (apparently aspergillosis) secondarily disseminated over the pleura. The latter morphological observation (disseminated spread of fungus over the pleura) has not been made very frequently and motivated the publication of this case.

Concluding the report, a short discussion on the classification of pulmonary Aspergillosis is attached.

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RECHERCHES EXPÉRIMENTALES SUR LES VARIATIONS DU CYCLE DE QUELQUES URÉDINALES SOUS L'ACTION DU CLIMAT ALPIN ET DU CLIMAT BORÉAL

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(18.XI.1960)

Le développement d'une Euforme dite Macrocyclique, comme *Puccinia violae,* comprend 5 types de spores: 1° une basidiospore germe reproducteur dont l'individualisation est précédée de la méïose; 2° une spermatie, germe gamétique; 3° une aecidiospore transmettant le germe sporophytique d'un hôte à l'autre; 4° une urédospore, multipliant le parasite sur le même hôte; 5° une probaside assurant la caryogamie et la conservation de l'espèce.

Les microformes dans la nature

D'autres „rouilles", attaquant des hôtes voisins, montrent un raccourcissement de leur Cycle: les hémiformes réduites à leurs urédos et à leurs téleutospores; les microformes telles que *Puccinia alpina* (récoltée par nous sur *Viola biflora* au Lac d'Oncet, Pyrénées — à 2.350 m) montrent le cycle réduit aux téleutospores. Ed. Fischer (1904), R. Heim (1928) et nous-même avons établi que les microformes réduites à leurs probasides sont plus nombreuses dans les régions arctico-alpines que dans la plaine. Ainsi 35 % des Urédinales à affinités alpines sont des microformes; les ubiquistes renferment 17 % de microformes. En Suisse 53,7 % des microformes appartiennent à l'étage alpin. Quelles sont les causes physiologiques de cette réduction du cycle?

Suivant Magnus les rouilles à cycle bref auraient plus de chances de se répandre sur des hôtes dont la végétation est raccourcie par le climat alpin (cf. Schneider (1906) et Ivanoff (1907)). Au contraire la forme à urédos serait adaptée à une longue période de végétation.

Remarquons pourtant que l'on rencontre des microformes non seulement dans la zone alpine, mais aussi parmi les plantes prématurément desséchées. Ce facteur nuit à la production des aecidiospores et des urédospores, mais favorise la formation isolée des basides (SMITH, 1904) (*Puccinia asparagi* D.C.). Même sur les hautes montagnes, à côté de microformes dominantes on reconnait certaines euformes (à cycle complet). Citons notamment:

A) espèces autoïques *Puccinia soldanellae* (D.C.) FÜCK sur *Soldanella alpina* (I, II et III) en Suisse (Mayor) et une grande partie de l'Europe;

Puccinia punctata LINK sur *Galium cometerrhizon* (I, II et III) (Pic du Campbielh — versant Sud au-dessus du col de Campbielh — 2.600 m, 14-IX-1958);

Uromyces armeriae (SCHLECHT.) LÈV. (I, II et III) (Pic Nègre) d'Envalira (2.600 m) 3-IX-1950.

B) espèces hétéroïques: *Melampsora alpina* JUEL S, I, sur *Saxifraga Jouffroyi* (*S. iratiana S. moschata*) Gourgs-blancs prés Luchon (2.800 m) 1-VIII-1948. S, I, sur *Saxifraga moschata* (Pic du Midi de Bigorre 2.830 m, 19-VII-1951);

II, III, sur *Salix pyrenaica* et *S. reticulata* (Ports d'Urets — Ariège — 2.500 m — 23-VIII-1950);

II, III, sur *Salix retusa* et *S. reticulata* (Pic du Midi de Bigorre 2.830 m, 18-VIII-1954);

Puccinia polygoni vivipari KARST. sur *Polygonum viviparum* (Cirque de Barroude, au-dessus d'Arragnouet — 2.350 m, 9-VIII-1949);

Phragmidium fusiforme SCHROET sur *Rosa alpina* (sous le Port de Campbielh — 2.300 m, 6-VII-1950).

La température, surtout celle de la nuit, influe aussi sur le développement des „rouilles". KURSANOV montra (1915—1917) que la croissance de *Triphragmium ulmariae* (SCHUM.) LINK sur *Spiraea ulmaria* était accélérée à l'Orangerie de Moscou plus que dans la nature. La suspension hivernale de la végétation de l'hôte retardait de 4 mois la croissance et la conjugaison nucléaire du parasite.

INVESTIGATIONS EXPERIMENTALES SUR LE CYCLE À DIVERSES ALTITUDES

Nous avons recherché les causes de l'abréviation du cycle des „rouilles" en les cultivant pendant 5 années consécutives:

1° au Pic du Midi de Bigorre (Jardin alpin 2.830 m),
2° à Pène-blanque (1900—2000 m),
3° à Bagnères de Bigorre (550 m),
4° à Toulouse (Jardin Botanique 80 m).

Plusieurs plantes alpines se prêtaient mal à l'expérimentation en plaine: *Potentilla nivalis, P. alchimilloides, Hutchinsia alpina, Salix herbacea* résistant difficilement aux chaleurs estivales, végétant d'une manière désordonnée, épuisant leurs réserves, mourant prématurément.

Les espèces montagnardes s'accommodant de telles modifications du climat avec le moins de désavantage étaient:

I) *Alchimilla pubescens*, *A. glaberrina* sensibles à l'attaque de *Trachyspora alchimillae* FÜCK.;

II) *A. hoppeana* réceptive à *Tr. melospora* (THERRY) SYDOW (des Alpes et Jura suisses) jamais rencontrée dans les Pyrénées.

La première, hémiforme, produisait successivement urédospores et probasides en abondance à l'étage montagnard.

Si les observations dans la nature étaient fondées, l'expérimentation aux hautes altitudes devait montrer une condensation ou une abréviation du cycle aux dépens des urédospores.

La seconde sous-espèce, microforme, produisant presqu' exclusivement des téleutospores (exceptionnellement des urédospores) on pouvait renverser l'expérience, le réchauffement des nuits en plaine accroissant la proportion des urédospores; ainsi nous recherchions si la variation provoquée des facteurs externes modifiait les rapports numériques des diverses catégories de spores d'espèces voisines, en choisissant comme sujet d'expérience des parasites où l'inégale proportion des spores dans la nature permet d'envisager la possibilité d'une rupture d'équilibre plus accessible aux variations expérimentalement provoquées. Enfin la végétation vivace des hôtes et de leurs parasites (dans les rhizomes) permettait de prolonger les expériences pendant plusieurs années au cas où un accommodat se manifesterait progressivement. Les ensemencements des akènes d'Alchémilles saines restèrent d'abord infructueux, malgré la diversité des substrats utilisés: terre, terreau, sable siliceux, verre pilé. Le séjour (15 jours) au frigidaire, la récolte à diverses époques (maturation), la stratification, la présence de mycorhizes, ne réussirent pas mieux. L'hybridation fréquente des Alchémilles européennes nous expliqua les malformations des graines. L'ensemencement d'un compost renfermant 2/3 de terreau, 1/3 de mélange de terre fraîche et de sable, à l'ombre et sous châssis, nous permit d'obtenir une douzaine de germinations d' *A. vulgaris* et davantage d'*A. wetteri*. Il fallut donc repiquer à plusieurs altitudes des pieds récoltés entre 750 m et 2.100 m.

Les techniques d'infestation consistaient:

1) en dépôt de spores dans l'eau pulvérisée sur les feuilles;
2) en dépôt de germes sur l'épiderme excorié;
3) insertion de fragments de limbes contaminés entre les plis des jeunes feuilles.

La contamination de deux Alchémilles vulgaires se manifesta un an après l'infestation expérimentale. La faible proportion d'urédospores pouvant germer (10 %) expliquait la contamination limitée. L'ensemencement sur les cotylédons fut peu efficace. Le contact étroit (pendant 48 heures) entre un pétiole parasité par la forme à urédos et celui d'une feuille saine permit d'infester cette dernière après une incubation de 52 jours.

Résultats obtenus

Les urédospores s'ouvrent d'autant plus tradivement que la station est plus élevée:

à Toulouse (80 m) dans la première quinzaine d'Avril;
à Bagnères (550 m) du 15 au 26 mai;
à Rimoula (900 m) du 5 au 10 juin;
à Pène-Blanque (1.900 m) le 25 juin;
au Jardin Botanique Alpin du Pic (2.830 m) du 3 au 6 Août.

Le facteur important est la température, une précocité plus grande marquant les années où la température diurne se relève à 8° — 10° C en l'absence de gelées nocturnes.

La localisation des sores est modifiée par l'altitude: ils se montrent plus clairsemés et plus nettement alignés près des nervures, sur les hautes montagnes (2.830 m) que vers 1.900 m, où ils recouvrent densément la face inférieure des limbes.

Une humidité persistante et élevée (71° à 100°)conditionne la fructification du parasite (corrélativement à une température suffisante). La déhiscence des sores est déterminée par la turgescence en fonction:

1° d'un afflux d'eau dans les feuilles, 2° d'une migration des glucides contrariée par le froid nocturne. (Lascombes (1952) a d'ailleurs montré que la migration des glucides de betterave est suspendue par ces gelées). Les écarts de température (14—26° C) entre la périphérie et le centre d'une touffe aux altitudes supérieures, expliquent la localisation des sores et leur ouverture sous les feuilles abritées contre une évaporation excessive.

Durée de la Sporogénèse

La durée respective des stades à urédos et téleutospores varie suivant les espèces: a) sous le rapport de la précocité; b) de la durée de la production de chaque type de spores. Sur *A. silvestris* se formaient des urédospores en juillet, époque où *A. glaberrima* ne portait plus que des probasides. Des races géographiques se manifesteraient sous ce rapport.

L'ordre de la succession des types de spores sur un même organe est généralement respecté. Une plante d'Alchemille en végétation active et hébergeant le mycelium pérennant peut former jusqu'en Octobre des urédos sur ses jeunes feuilles, mais presqu'exclusivement des probasides sur les feuilles bien développées.

Répartition des spores dans les sores

Les urédospores sont formées typiquement dans les sores dont la confluence détermine l'aspect de revêtement hypophylle incrustant. En plaine les probasides prennent généralement naissance dans des sores distincts des urédos, plus exigus, plus rarement coalescents. A haute altitude (2.830 m) les sores mixtes communs aux deux catégories de spores se montrent plus nombreux qu'en plaine. Exemple: le 18 Août 1955 (après 4 ans de parasitisme sur la même plante au sommet du Pic du Midi), les sores mixtes renfermaient 30,9 % d'urédospores, 63,6 % de probasides et 5,3 % d'amphispores sphéroïdales intermédiaires. Le nombre des sores mixtes s'accroît d'année en année ainsi que la tendance à la condensation du cycle réduit (urédospores + téleutospores). Cependant nous n'avons observé que rarement la disparition du stade à urédospores (qui a manqué une fois) dans nos cultures comparées, à haute altitude pendant 5 ans. La durée du stade à urédospores est seulement abrégée; sa disparition au cours d'une adaptation progressive est d'autant plus improbable que la végétation des Alchémilles ne dépasse guère 5 ans.

L'expérience inverse consistait à cultiver, de 80 m à 2.830 m, la race microcyclique alpestre *T. melospora* pour rechercher s'il se différenciait une proportion plus élevée d'urédospores en plaine qu'en montagne. Or jamais elle n'a produit plus de 1 % d'urédospores à Toulouse.

L'équilibre des caractères observés chez ces deux races ne peut donc être modifié dans les conditions de l'expérience: ces races ne peuvent passer ainsi de l'état d'hémiformes à celui de microformes en les cultivant à de hautes altitudes.

Par quel processus naissent les microformes?

A Abisko (Laponie) sous le cercle polaire, RYTZ (en 1927) observa, sur les feuilles d'*A. vulgaris*, des pustules presqu'exclusivement téleutosporifères (telles que celles de *Tr. melospora* parasite sur *A. hoppeana* alpestre). D'autres feuilles d'*A. vulgaris* étaient recouvertes de sores à urédos et à probasides de *Tr. alchimillae.* Nous supposons que cette dernière a subi une mutation par perte de la propriété de produire des urédos. Elle persisterait concurremment avec l'hémiforme; leur coexistence s'expliquerait par l'absence de spermaties, rendant plus difficile la fécondation croisée.

IVAR JÖRSTAD (1951) a observé en Islande 29 % de téleutospores dans l'hybride *A. filicaulis* × *A. vestita* (300—400 m) et une proportion de 66 % chez *A. glomerulans* (760 m) dans la zone alpine. Il semble donc que le froid nocturne provoque une mutation à haute altitude (dans la région Scandinave). La comparaison entre:

1° nos résultats expérimentaux relatifs à la durée restreinte de la sporogénèse et à la condensation en sores mixtes;

2° les observations de RYTZ concernant les microformes;

3° celles de JÖRSTAD montrant la décroissance dans la production des urédospores, nous amènent à envisager un schéma naturel de la variation spécifique aux basses températures. L'adaptation aux climats froids et humides (alpin ou circumpolaire) ne déterminerait pas d'emblée la réduction du cycle, chez l'espèce perennante, mais la condensation en sores plus exigus sans modification concomitante des caractères spécifiques. La mutation brusque pourrait seule faire apparaître un nouvel équilibre spécifique, caractérisé par la disparition des urédospores qui définit une forme microcyclique. Celle-ci coexiste quelque temps dans la nature avec l'hémiforme. Il est possible que la microforme (mieux adaptée au froids nocturne que l'hémiforme) finisse par se maintenir seule, en raison de son cycle raccourci.

CONCLUSIONS

1° La proportion des Urédinales à microformes est plus élevée sous les climats alpins et arctiques qu'aux basses altitudes. Cependant 28 % des „rouilles alpines" produisent encore des urédospores.

2° Nous avons recherché expérimentalement le déterminisme de la formation des urédos et des téleutospores, en comparant en plaine et en montagne, 2 sous-espèces vivaces de *Trachyspora*: l'une hémiforme, *T. alchimillae* parasite d'*Alchimilla vulgaris*; l'autre microforme, *T. melospora* sur *A. hoppeana*, cultivées pendant 5 années consécutives de 80 m à 2.830 m d'altitude.

3° Le stade à urédospores est abrégé sous le rapport de la durée: les urédospores tendent à se condenser en sores mixtes sous l'action du climat alpin.

4° La proportion élevée, 99 %, des probasides chez *T. melospora* n'a pas subi de modifications aux basses altitudes.

5° Nous avons comparé nos résultats expérimentaux, concernant la condensation des sores, avec les observations relatives aux microformes et à leur coexistence avec des hémiformes sous le cercle polaire.

6° Nous avons proposé une conception de la variation spécifique, dans ce groupe d'Urédinales, sous l'action du refroidissement nocturne; l'adaptation ne provoquerait pas en géneral une abréviation du cycle chez les espèces pérennantes.

La mutation fait apparaître sporadiquement un nouvel équilibre spécifique qui tendrait à faire disparaître la forme à urédos.

La microforme coexisterait quelque temps avec l'hémiforme. Cette dernière, moins bien adaptée aux climats froids, finirait par disparaître.

SUMMARY

VARIATIONS OF THE CYCLE OF SOME "RUSTS" UNDER THE ALPINE AND BOREAL CLIMATES

Some of the spore forms are lacking in many species, especially in alpine plants. We have sought for the causes of this abbreviation

of development by means of compared experimental cultures in low plains and on high summits simultaneously. We have chosen two closely related species of "rusts" parasitic on Alchimillae common in the mountains (Pyrénées):

a) one of them, *Trachyspora alchimillae* being a hemiform, deprived of spermatia (or pycnospores) and of aecidiospores;

b) a second species, *Trachyspora melospora*, a microform, the teliospores of which are nearly the sole reproductive form (99 %). After five years of experiments, we have observed the abbreviation of the uredo stage, which was seldom absent even at the highest level (9.400 feet). More often, both kinds of spores (uredos and teliospores) were formed side by side in mixed sori. Mycelium being perennial in the rhizome, infection occurs every spring on young leaves, the petioles of which are growing in length abnormally, and leaf-blade dwarfing generally.

We have compared experimental results on high mountains with observations in the northern region of Europe (Finland, Iceland) — (RYTZ, JÖRSTAD, MORQUER).

As a matter of fact, a mutation is induced under the polar circle: urediospores disappeared in several host plants. At any rate, the microcyclic mutant and the hemiform parent strain continue to exist side by side. Moreover the ratio of microcyclic mutant individuals increases with the altitude in the same region.

Cold nights and winter are more likely to induce mutants (with reduced cycle) which are more stable than the hemiform in alpine and boreal environment.

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ELICITATION OF PERFECT ORGAN OF FRUCTIFICATION IN SABOURAUD'S FORM-GENUS MICROSPORON (PRO PARTE) BY MEANS OF SYMBIOSIS WITH B. WEIDMANIENSIS BENEDEK, 1938

by

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(with 17 figs.)

(9. III. 1961)

> *Nonumque prematur in annum*
> *Horace, Ars Poetica, v.* 388

Almost two decades ago I published my findings on unilateral stimulation of *Microsporon Audouinii* by an incidentally found bacillus, *B. weidmaniensis* (2). At that time I expressed my hope that the final paper concerning the formation of perfect organ of fructification in Sabouraud's form-genus *MICROSPORON* (pro parte) under the impact of the symbiosis with *B. weidmaniensis* would soon follow. The good intentions were, however, nullified by the outbreak of the second World War and the following prolonged war service. In the post war period other microbiological problems (permanent endoparasitism) so thoroughly occupied my mind and time, that the promised publication of my results have time and again been delayed up to the present.

In order to connect the past with the present, I herein give a summary of the findings laid down in the first portion of this communication.

A. Discovery of the perfect organ of fructification in Sabouraud's form-genus Microsporon (pro parte)

During the comparative study in the form-genus *Microsporon*, Conant (7) found that *M. Audouinii*, in sharp distinction from *M. lanosum*, does not vegetate at all on rice medium. Years before, during the study of a newly discovered species of *Microsporon*, *M. Stilliansis* Benedek, 1938, (1), making comparative investigations with *M. Audouinii* and several animal species of *Microsporons* on rice medium, I readily substantiated the observation of Conant as to the difference of viability of human and animal species of *Microsporons* on rice.

As a rule every freshly isolated strain of *Microsporon* went through rice medium in the process of identification in my laboratory because that at present is the only sure way to exactly differentiate human and animal species of *Microsporons*. During these routine examinations a strain of *M. Audouinii* was freshly isolated from hair stumps of a Negro boy. In the primary culture on Difco Sabouraud glucose agar a bacterial growth, conspicuous by its mustard yellow color, was vegetating along with the fungus. The young primary colony of this strain of *M. Audouinii* grew so intimately intermingled with the yellow bacterial growth that a separation of the two microorganisms for the purpose of taking an inoculum for rice medium was practically impossible.

Thus, the inoculum transplanted to rice was transmitted along with the yellow bacterial growth. About five days later I observed against any expectation that this strain of *M. Audouinii* had begun to grow and later, yielded a luxuriant growth on rice medium. Microscopic examination of the fungal growth revealed (*a*) the mustard yellow bacterial growth vegetating along the mycelium, (*b*) *M. Audouinii* producing many giant septate and non-septate spindle spores, contrary to its attitude on any of the routine mycological mediums where macroconidia usually are sparse or completely absent.

Further investigation showed that this vital stimulation is a permanent effect to be elicited at any time and with any strain of *M. Audouinii*. It makes no difference whether the rice medium is simultaneously inoculated with *M. Audouinii* and the bacterial strain; or the rice medium is first inoculated with the bacterial growth and any time (save exsiccation) thereafter, the inoculation of *M. Audouinii* follows; or the rice medium is first inoculated with *M. Audouinii* and then up to 12 days afterwards the fungus inoculum is infected with the bacterial growth.

The newly discovered bacterial microorganism is characterized by rod-shaped cells, production of a mustard-yellow pigment on all mediums, by extreme polymorphism and definite spore formation. It is non-motile, aerobic, gram-negative and does not liquefy gelatine.

The unilateral vital stimulation of *B. weidmaniensis* BENEDEK, 1938 on *M. Audouinii* induced not only a luxuriant vegetation on rice medium with rich production of large spindle spores, but it caused this imperfect fungus to produce cleistothecia in extreme abundance. *B. weidmaniensis* is, thus, the first agent by means of which a medically important hyphomycete as the *M. Audouinii* could be induced to produce cleistothecia. *B. weidmaniensis* was expected to have great heuristic value as a biological tool in investigation and in attempts to induce a higher form of fructification in different species of Fungi imperfecti. It was also hoped that this discovery might arouse interest in and lead to a systematic investigation of the value of different bacteria as unilateral vital stimulating agents in connection with hyphomycetes for the production of a higher form of fructification. (†)

During the course of the following two decades my publication elicited but little interest. In the literature I found only one communication on the subject by HAZEN. The growth of *M. Audouinii* with production of macroconidia on polished rice grains infected with *B. weidmaniensis* had been confirmed by the late RHODA W. BENHAM (5). HAZEN (9) found that with the addition of *B. weidmaniensis* to rice grains, all of the cultures (9 strains altogether of *M. Audouinii*) produced macroconidia in number comparable to those on the yeast-extract medium. She assumed these findings support the idea that the substance produced by the bacillus may be in the nature of vitamins.

B. MATERIAL AND METHOD

In the experiment to elicite a higher organ of fructification, nine freshly isolated strains of *M. Audouinii* and five strains of *M. lanosum*, respectively, have been used. All the strains originated from tinea capitis with typical fluorescence in Wood light. The epilated hairs revealed the characteristic, mosaic-like dense spore sheath around the hair. The primary differentiation between *M. Audouinii* and *M. lanosum* was made by means of polished rice medium.

Erlenmeyer flasks of 50 ml volume contained 5 g of commercially available polished rice grains and 20 ml of physiological NaCl. They were sterilized in steam for an hour.

Every single experiment with a single strain was carried out in duplo. Three rice mediums were used each time: no. 1 remained uninoculated; no. 2 was inoculated with *B. weidmaniensis* and, no. 3 with *Microsporon* and *B. weidmaniensis*. These cultures remained at all time at room temperature. No. 1 remained sterile all the time, while no. 2 revealed only the growth of *B. weidmaniensis*.

The microcultures (hanging drop cultures) were widely used by means of the Van Tieghem cell. Hollow slides and cover glass were always flame-sterilized. Each time, two slides with four microcultures were prepared. Sterile, crushed rice grain particles were deposited on the cover glass. Here again, one microculture contained only crushed rice grain particles; the second, rice grains and *B. weidmaniensis*, while the third and fourth contained rice grains, *B. weidmaniensis* and *Microsporon*.

Sections from cultures, whether grown on rice, barley or agar

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(†) I was also successful in producing perithecia in *Aspergillus flavus* by means of *B. weidmaniensis* on rice medium.

mediums, (Sabouraud's maltose and glucose agar, Difco Bacto brand) were prepared as follows: Cultures of different ages were used for histological purposes. The surface of the culture, — agar or grain, was covered with the following fixative for several days,

Lavdowsky's formol-acetone-alcohol:

formol 10 %	10 parts
alcohol 95 %	50 parts
glacial acetic acid	2 parts
acetone	3 parts
dist. water	40 parts

followed by 70 % alcohol and routine histological technic. With a round, crown-sharp small potato cutter, pieces of about 1 cm in diameter were cut from the medium, agar or grain. From these pieces, frozen sections were made. 1) Concerning stains hematoxylin-eosin gave the best results. PAS and Hotchkiss-MacManus stains were also tried, but due to their rapid overstaining, they yielded unsatisfactory results, pictures being blurred, lacking finer detail.

C. Formation of the organs of fructification, perfect and imperfect

After a week, the whole surface of the rice medium, due to the scattered inoculum, was covered with a white velvety growth. At the end of the second week, pinpoint to pinhead size tiny white to grayish-white round bodies appeared scattered on the mycelial surface. They were just at the extreme limit of visibility to the naked eye but could be well visualized by means of a simple magnifying glass. In taking samples from different points of the surface one could see typical pointed spindle-shaped, thick-walled, warty macroconidia and the round to oval, light grayish-brown perithecial bodies. There was no difference in the organs of fructification, perfect or imperfect, as to whether the inoculum came from *M. Audouinii* or *M. lanosum*. The imperfect organ of fructification (macroconidia) which was present together with the perfect organ of fructification, in later subcultures became more and more sparse, to eventually disappear completely leaving only the cleistothecia in the culture.

From this point on, the presentation of my material follows in a pictorial manner. The legends are exhaustive and points of interest well marked.

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1) The histological preparations of the mycological material were carried out with great art and accuracy by Miss Avis Gregersen, F.B.P.A., at that time, in Chicago and, presently at the Department of Dermatology, U.S.C. at Los Angeles. My great appreciation and gratitude is due Miss Gregersen for her most valuable support of my work.

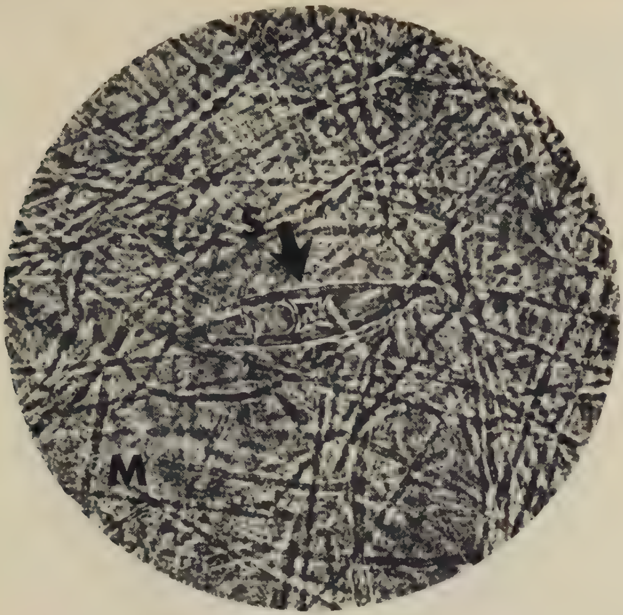

Fig. 1. *Veronaia* (*Microsporon*) *Audouinii*, strain 137/2, grown on rice medium inoculated with *B. weidmaniensis* BENEDEK at room temperature, 24 days old. Culture particle mounted in chlorallactophenol. A mat of sterile mycelium (M) with typical macroconidia (S)/fuseau, spindle spore. X248, 2.4x ¹) ²)

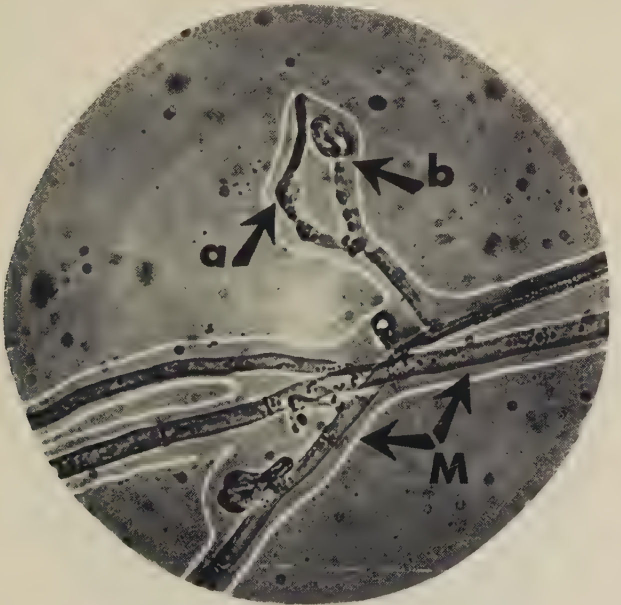

Fig. 2. *Veronaia* (*Microsporon*) *Audouinii*, strain 137/2, hanging drop culture in van Tieghem cell on polished rise inoculated with *B. weidmaniensis* BENEDEK. Development of a cleistothecium: a and b sexual branches; M sterile mycelium. Homothallic. Culture 8 days old. X248, 2.4x.

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¹) The figures indicate: "magnification" X248, "enlargement" 2.4x. This abbreviation is used throughout this paper.

²) All the photographs and photomicrographs have been taken by the author, except for photomicrographs in Figs. 11 and 12 which were obligingly made by Prof. O. VERONA, Pisa, Italy.

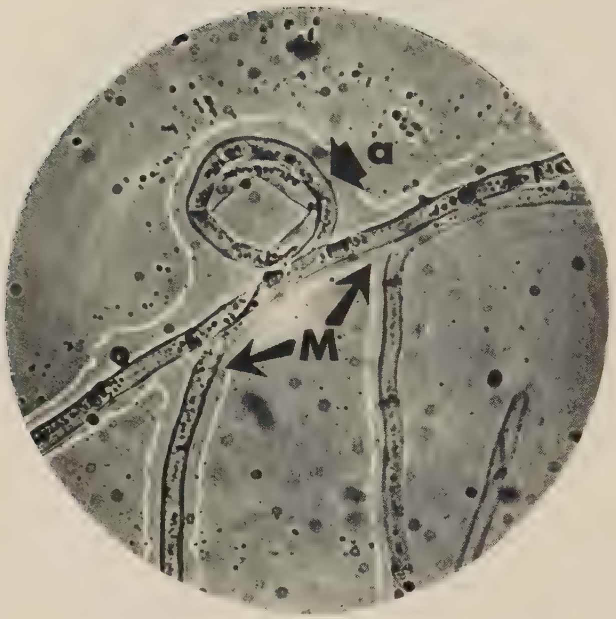

Fig. 3. *Veronaia* (*Microsporon*) *Audouinii*, strain 137/2. Data the same as in Fig. 2. (a) coiling of a sexual branch; M sterile mycelium. X248, 2.4x.

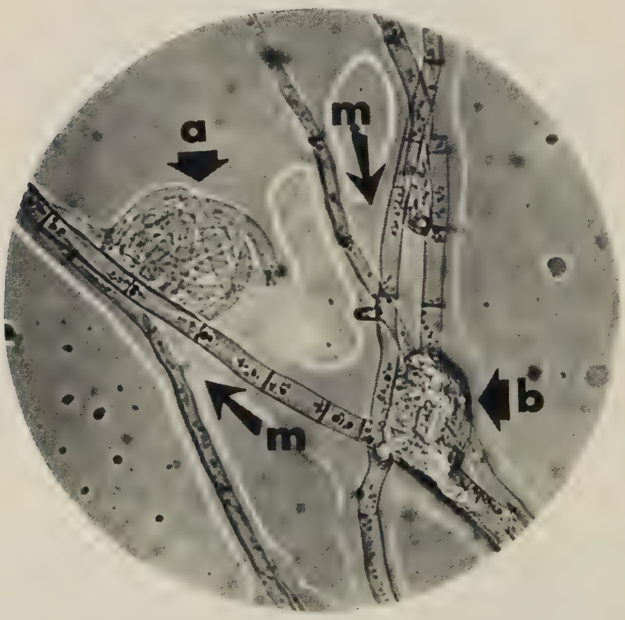

Fig. 4. *Veronaia* (*Microsporon*) *Audouinii*, strain 137/2. Data the same as in Fig. 2. a and b progressive coil formation; m sterile mycelium. X248, 2.4x.

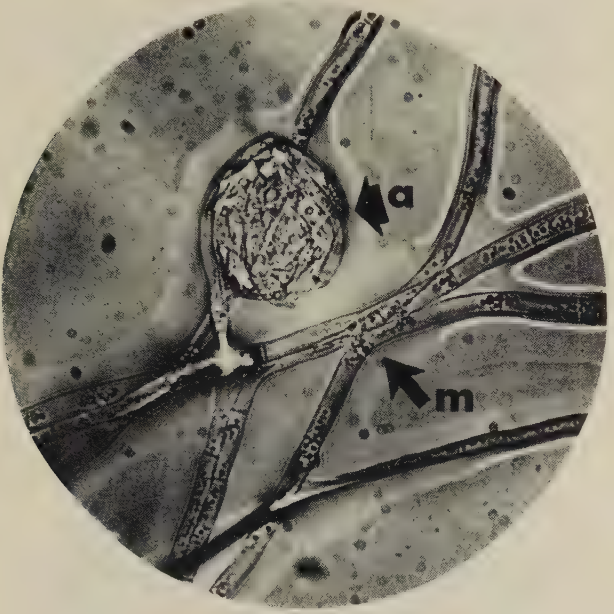

Fig. 5. *Veronaia* (*Microsporon*) *Audouinii*, strain 137/2. Data the same as in Fig. 2. (a) the last phase of the coil formation is completed, the early cleistothecium established; m sterile mycelium. X248, 2.4x.

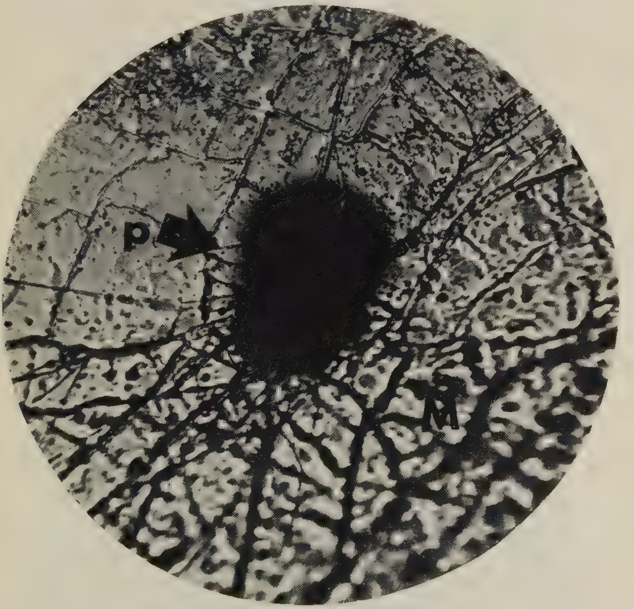

Fig. 6. *Veronaia* (*Microsporon*) *Audouinii*, strain 137/2. First subculture on Sabouraud maltose agar. Cleistothecium is now fully established. Culture 15 days old. Photo taken through the glass wall of the test tube. p cleistothecium, M sterile mycelia. X248, 2.4x.

The Figs. 7 to 15, are photomicrographs of frozen sections through the culture medium on which the fungi were grown. The sections were stained with hematoxylin eosin.

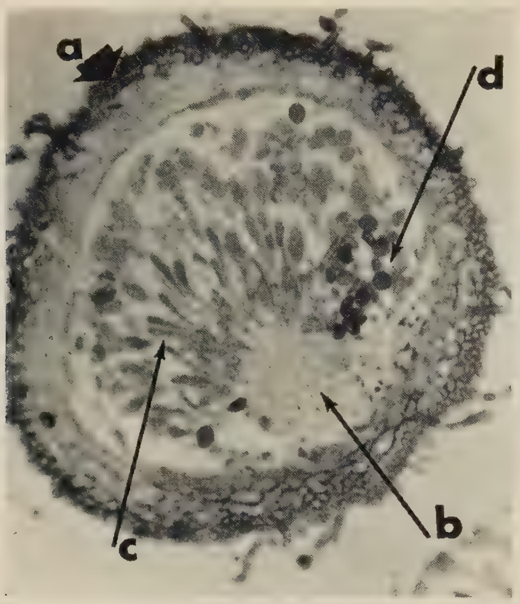

Fig. 7. *Veronaia* (*Microsporon*) *Audouinii*, strain 144, subculture grown on plain polished rice at room temperature, 2 weeks old. Cross section of a cleistothecium showing (a) peridium (observe its thickness, compared with the peridium grown on Sabouraud maltose agar, Fig. 8), (b) ascogon, (c) club-shaped ascogenous hyphae, (d) ascospores, in progressive phases of their development. X320, 2.9x.

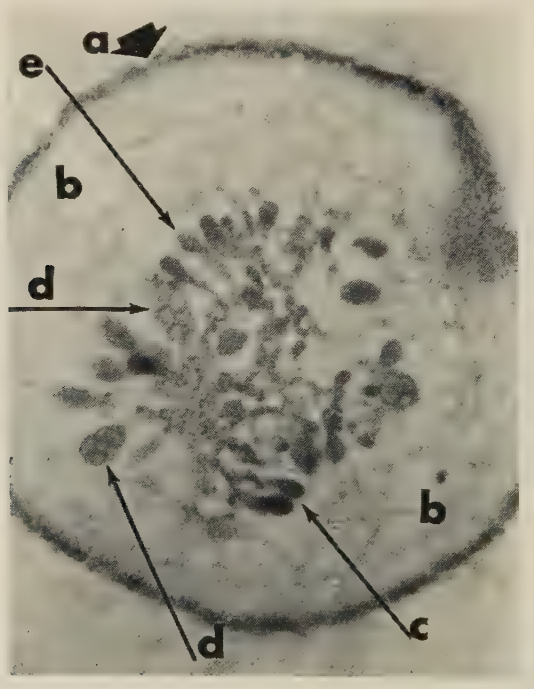

Fig. 8. *Veronaia* (*Microsporon*) *Audouinii*, strain 137/2, subculture grown on Sabouraud maltose agar, at room temperature, 1 week old. It shows (a) peridium (much thinner than on rice medium), (b,b) a fine net of ascogenous hyphae, (c,e) progressive swelling of the club-shaped ends of the ascogenous hyphae, just before they give rise to the ascus with an extremely thin wall, (d) ready asci with eight spores (the spores are held together for awhile by a slimy mass). X400, 2.9x.

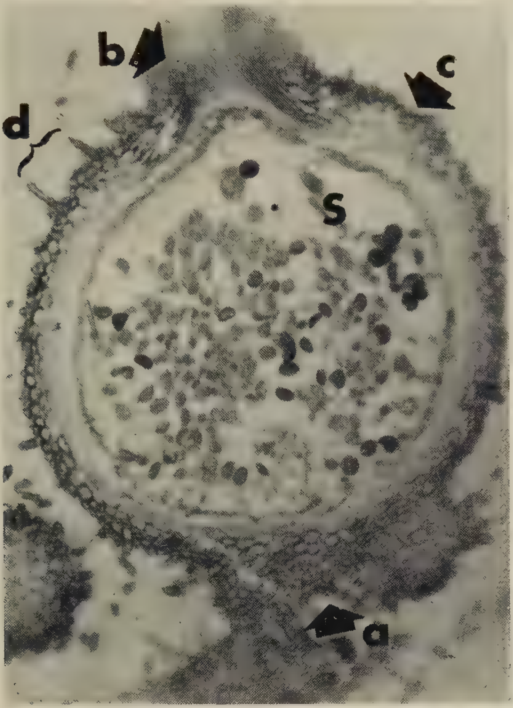

Fig. 9. *Veronaia* (*Microsporon*) *Audouinii*, strain 144, grown on plain rice (subculture) at room temperature, 2 weeks old. (a) cleistothecium arising from the stroma, (b) a secondary cleistothecium arising on the top of the first one, (c) thick wall of the peridium, (d) secondary ascogenous hyphae; (S) ascospores within the cleistothecium, grouped or already single, in different stages of maturity. X320, 2.9x.

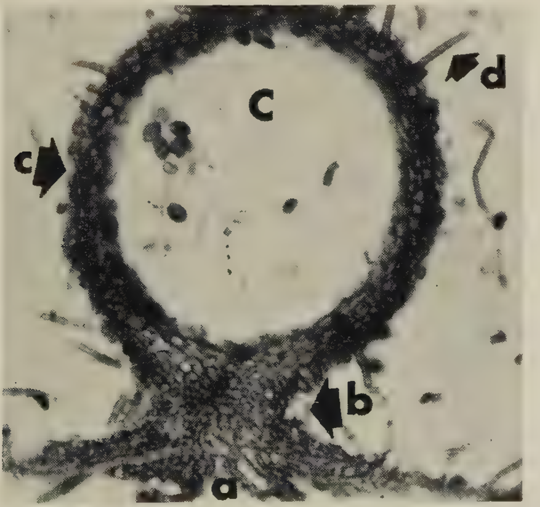

Fig. 10. *Veronaia* (*Microsporon*) *Audouinii*, strain 144, data the same as in Fig. 9. It shows the perfect cross section of a cleistothecium arising at (a) from the stroma, on a pseudostalk (b), (c) thick peridium and within the cavity of the cleistothecium (C) few ascospores with shreds of the disappearing ascogenous hyphae, (d) secondary ascogenous hyphae. (During the cutting of the section the rest of the ascospores were wiped outside.) X320, 2.9x.

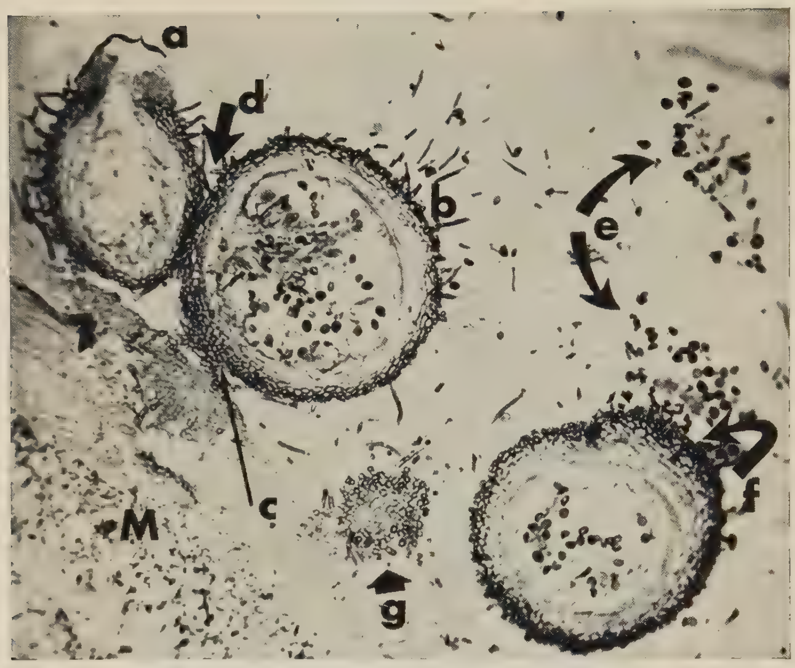

Fig. 11. *Veronaia* (*Microsporon*) *Audouinii*, strain 144; data the same as in Fig. 1. Cross section through a group of cleistothecia. (a) is not an ostiole! This is the site where the pseudostalk of a tertiary cleistothecium had broken off; (b) a primary cleistothecium rising at (c) from the stroma, sessile. (d) the site of the production of a secondary cleistothecium from the ascogenous hyphae of the peridium; (e) scattered ascospores due to the cutting of the section; (f) the pseudostalk of a cleistothecium cut away from the stroma; (g) a young, small cleistothecium; M sterile mycelium. X140.

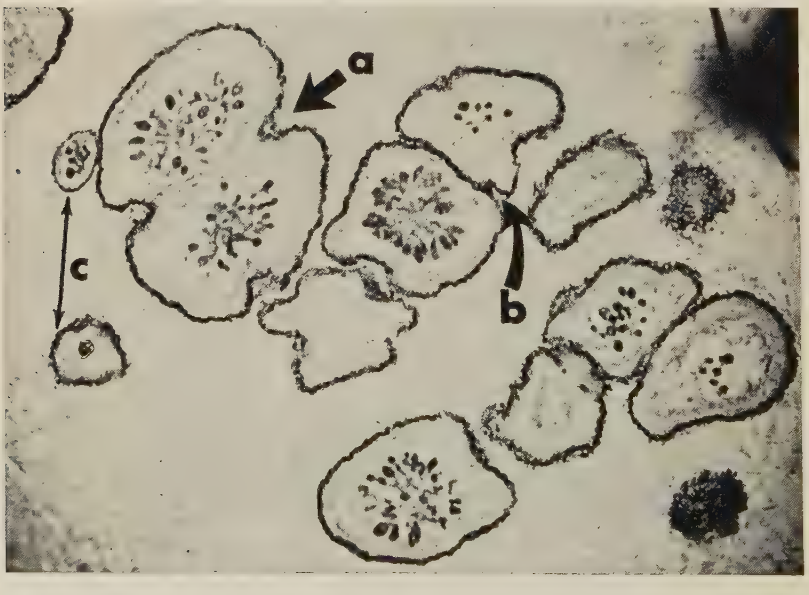

Fig. 12. *Veronaia* (*Microsporon*) *Audouinii*, strain 137/2, subculture grown on Sabouraud maltose agar at room temperature, 1 week old. Cross section through an aggregate of cleistothecia. Observe the thin peridium of the cleistothecium! (a) double cleistothecia without separating peridium in between; (b) triple cleistothecia with separating peridium in between; (c) young primary and secondary cleistothecia. X140.

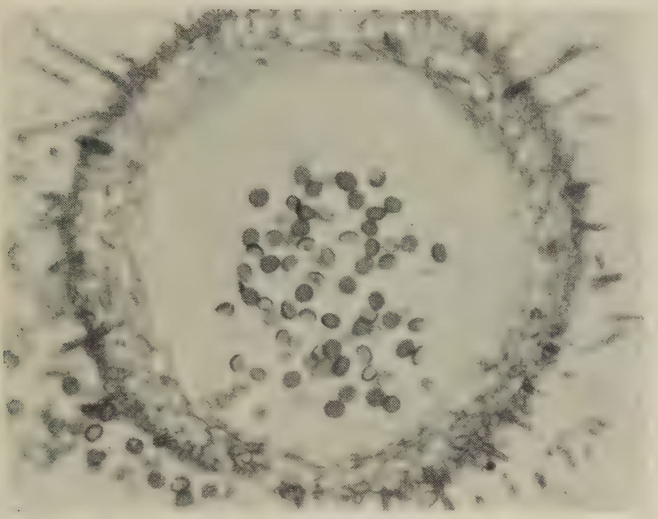

Fig. 13. *Veronaia (Microsporon) felinea,* strain 154b, subculture on plain *rice,* 6 weeks old at room temperature. It shows the cleistothecium with the "braided" peridium of 7 or 8 cell layers with the loose, torn off ascogenous hyphae leading to other cleistothecia; inside mature ascospores. X320, 2.9x.

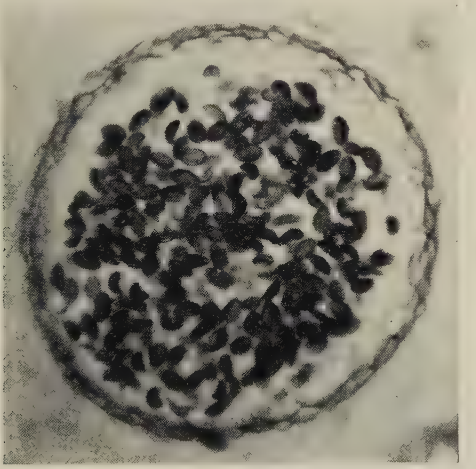

Fig. 14. *Veronaia (Microsporon) felinea,* strain 154b, subculture on *barley,* 5 weeks old at room temperature. Compare peridium of cleistothecium in Fig. 13! On barley, as on Sabouraud matose agar, the "braided" peridium is much thinner (2—3 cell layer), surface is smooth without secondary, interconnecting, ascogenous hyphae. X320, 2.9x.

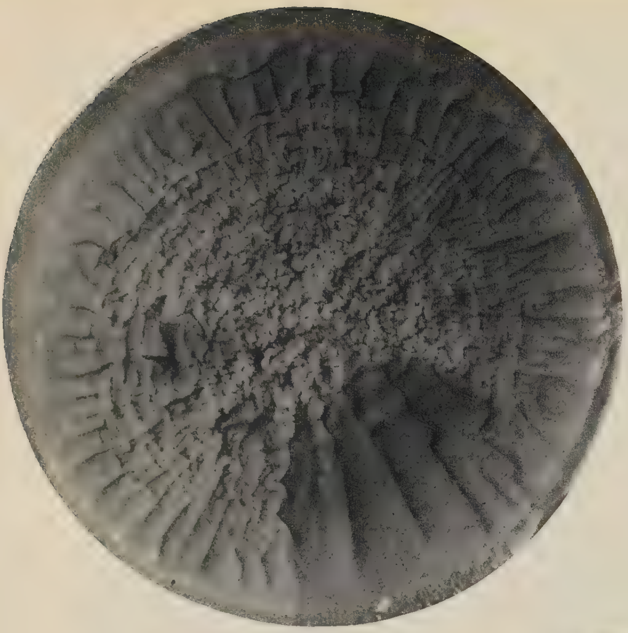

Fig. 15. Giant culture of *Veronaia Castellanii* Benedek, nov. spec. 1960 on Sabouraud's glucose agar. In the right lower quadrant the colony is dissociating. 4 weeks old, at room temperature.

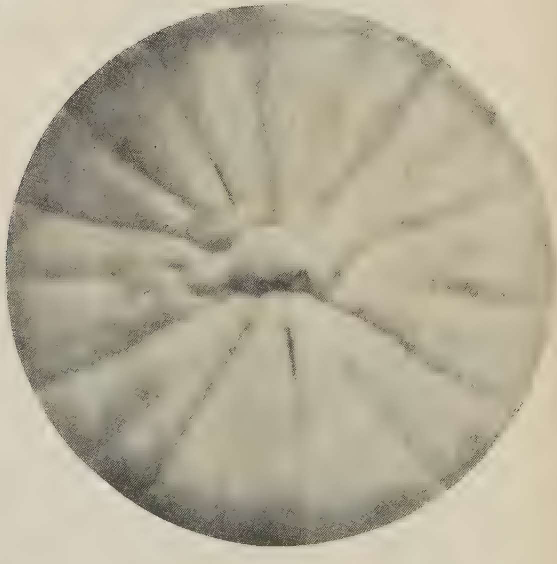

Fig. 16. Giant culture of *Veronaia Castellanii* Benedek, nov. spec. 1960 on Sabouraud's maltose agar. 4 weeks old, at room temperature.

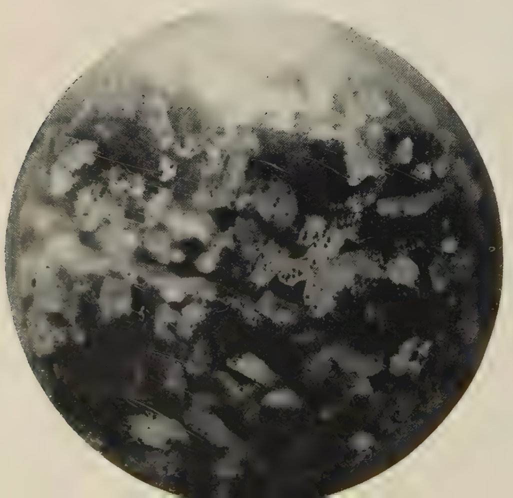

Fig. 17. Culture of *Veronaia Castellanii* Benedek, nov. spec. 1960 on plain polished rice medium. 4 weeks old, at room temperature. (Photograph is taken through an enlarging glass. Inspect it with a magnifying glass to see the cleistothecia.)

D. OBSERVATION OF TWO STRAINS OF MICROSPORON SPONTANEOUSLY PRODUCING PERFECT ORGANS OF FRUCTIFICATION IN THE PRIMARY CULTURE, WITHOUT ANY FORM OF STIMULATION

In November of 1940, only a few weeks apart, in short succession, two cases of tinea capitis had been observed in two small Negro boys, unrelated, five and seven years of age, respectively. Clinically, they showed several classical, grayish-dusty patches with many broken off hairs. They showed characteristic fluorescence for *Microsporon* in Wood Light. Microscopically the hairs revealed the typical dense spore sheath surrounding the hair in mosaic arrangement. The spores measured 3 to 5 μ in diam., mycelial threads 3 to 3.5 μ. They carried the accession number, 147 and 148.

Since both strains were identical, their cultural characteristics can be described together in a condensed manner.

Sabouraud glucose agar: Primary cultures became positive in 72 hours, the earliest; after five days all the inoculums (selected under Wood Light) have been found positive. On the sixth day the colonies measured 5 to 6 mm in diam. On the eighth day the colony reached 3 cm in diam. It appeared grayish-white in color, the surface was shiny and waxy. Radial folds ran from the center to the periphery. They were interrupted by a concentric fold, about 8 to 10 mm from the center. Later on, the primary folds showed further folding so that the whole surface became vermicular or better, cerebriform. On the sixteenth day the colony reached 8 cm in diam. The whole surface looked vermicular, except for the periphery where the colony ran out into a fine flat depressed aureola about 5 mm in diam.

Sabouraud maltose agar: The growth characteristics are the same. However, the colony is flat and much less cerebriform.

Difco Wort agar: The color is buff, surface cerebriform-vermicular, glabrous, shiny, waxy, not unlike a colony of *Achorion Schoenleinii*.

Natural mediums: A) potato: colony flat, cerebriform or warty, mouse-gray, velvety; B) carrot: identical with "A"; C) rice: in 3 to 5 days the whole surface is covered with a white loose, cottony growth.

On all mediums, agar or natural, within one to three weeks the same perithecial bodies appear on the surface of the colonies as already described in connection with the unilateral stimulation.

These strains show an extremely dense texture of mycelia; mycelia septate; homogenous or granulated; intercalary and terminal chlamydospores as well as arthrospores were present. The size of the chlamydospores are variable, between 7.5 μ by 15 μ to 15 μ by 30 μ.

E. TAXONOMIC POSITION OF THE FUNGUS STRAINS 147 AND 148

The clinical appearance of tinea capitis in the patients from whose hairs these strains have been cultured were exactly the same as those cases in which the culture ordinarily yielded *M. Audouinii* or *M. lanosum*. They gave the typical fluorescence under Wood light. The hairs under the microscope showed characteristic, dense spore sheath in typical mosaic arrangement.

The only difference was in the fact that these two cases yielded spontaneously in the primary culture a perfect organ of fructification along with the conidial (imperfect) fructification. The microscopical structure of the fungus strains, nos. 147 and 148, was by spontaneous development exactly the same as was found in *M. Audouinii* and in *M. lanosum* on rice under unilateral stimulation by the *B. weidmaniensis*. The production of macroconidia, the structural development of the perfect organ of fructification were identical. The conidial fructification (macroconidia) here also disappeared in later subcultures. Thus, the repetition of these details is not necessary.

In strains nos. 147 and 148, for the first time in the history of medical mycology, the long sought after perfect form of the form-genus *Microsporon* (pro parte) [1]) have been found.

Due to the specific developmental and structural characteristics of the perfect organ of fructification in the form-genus *Microsporon* (pro parte) the creation of a new genus became necessary. In honor of one of the foremost Italian phytopathologists and mycologists, I dedicate this new genus which is a landmark in the development of medical mycology to Prof. ONORATO VERONA of Pisa. Furthermore, it is a special privilege, to dedicate the type-species of the newly established genus, *Veronaia*, to the grand old man of world renown of medical mycology, Prof. ALDO CASTELLANI of Lisbon as *Veronaia Castellanii*.

Taxonomic position of VERONAIA Benedek, gen. nov.

ASCOMYCETES
- Ordo I. Gymnoascaceae
  - Family 3. Gymnoasci
    - Genus I. *Gymnoascus*
    - Genus II. *Ctenomyces*
- Ordo II. Perisporiaceae
  - Family 2. Aspergillales (Eurotiaceae CIFERRI)
    - Genus I. *Aspergillus* MICHEL
    - Genus II. *Penicillium* LINK
    - Genus III. *VERONAIA* BENEDEK, gen. nov.

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[1]) "pro parte" means that not ALL "species" of *Microsporon* do necessarily belong to the new genus, *VERONAIA*. Current investigations in progress have already revealed that other "species" of *Microsporon*, lumped together due to a common feature of macroconidia formation, do belong to entirely different genus or genera.

Dichotomic key of the species of the new genus VERONAIA Benedek:

A) Growth of colony fairly rapid; colony glabrous, waxy or velvety; color dependent on the carbohydrate in the nutrient medium: grayish-white to yellowish-green; pluriseptate macroconidia with pointed ends fairly abundant, microconidia rare or absent; production of cleistothecia abundant spontaneously on artificial and natural nutrient mediums.

VERONAIA Castellanii BENEDEK (type species)

B) Growth of colony slow, color white to beige, somewhat powdery. Macroconidia septate, pointed, fairly rare, rudimentary, microconidia usually absent.

VERONAIA (*Microsporon*) *Audouinii* GRUBY

C) Growth of colony rapid, woolly or cottony, white. Macroconidia pluriseptate, abundant, pointed, microconidia rare or absent.

VERONAIA (*Microsporon*) *felinea*, FOX and BLAX

Description of the new genus and type species:

VERONAIA BENEDEK, gen. nov., 1960
(Ascomycetes, Perisporiaceae, Aspergillales)
VERONAIA Castellanii BENEDEK, 1960 (type species)

Cleistotheciis globosis vel ovoideis vel piriformibus, singulis vel aggregatis, pseudoparenchymatosis, homothallicis, bruneis vel fuscis. Cleistotheciis parvis magnitudine ca 60 μ in diam., illis magnis ca 150—195 × 300 μ, sessilibus vel pseudo-pedunculatis. Hyphis sterilibus hyalinis, 1.5 μ — 2.0 μ; hyphis peridii valde ramosis, septatis, luteis vel bruneis, intertextis, 3.75 μ latis. Hyphis peridii ascogeniis altra cleistothecia aggregata frequenter producentibus. In mediis nutrientibus naturalibus (oryza, carota, patata) hyphis crassis peridii cleistotheciorum altris peridii hyphis semper emittentibus, tamen in agaro maltosato et in medio hordei nutriente muris peridii cleistotheciorum semper levibus. Hyphis ascogeniis ascogonio erumpentibus gracilissimis, ascosporis modo enorme formantibus; ascis octosporis, ascorum muris evanescentibus; ascosporis conglobatis, globosis vel ovalibus, hyalinis vel luteis, ca 7.5 μ latis. Postea structuris cleistotheciorum interioribus (hyphis ascogeniis gracilissimis) evanescentibus et ascosporis replentibus. Muris cleistotheciorum rumpentibus et ascosporis liberantibus.

Hab. in pilis hominis quibus folliculitem depilantem affert, status conidicus eodem consociato, ex cultura in auctoris laboratorio, Chicago, Illinois, U.S.A.

St. conidicus: Coloniis (in agaro glucosato Sabouraud) initio punctiformibus, dein diffusis, levibus, applanatis, disciformibus, radiis circumdatis, postremo luteolis, vel virido-ochraceis. Hyphis sterilibus, 1.5 — 2.0 μ latis, rectis, levibus, hyalinis, septatis. Hyphis fertilibus erectis vel subdecumbentibus, simplicibus, chlamydosporis, fusiformibus, multilocularibus, 5—7-septatis, 56—92 μ longis, 12—20 μ latis, deinde granulosis, solitariis, raro racemosis, hyalinis. Conidiis (aleuriis) absentibus, chlamydosporis fusiformibus raris, postremo elapsis in cleistotheciorum presentia.

Cleistothecia, round, slightly oval or pear-shaped, single or grouped, brown to black colored, pseudoparenchymatous, homothallic. While small and young, they measure about 60 μ in diam., when older and larger, they attain the size of 150 by 300 μ, 300 by 195 μ. They are sessile on the stroma or pseudostalked. Sterile hyphae are hyaline, 1.5—2.0 μ, perideal hyphae (ascogenous hyphae) are brown, 3.75 μ, both are septate. The peridium emits ascogenous hyphae which, in turn, produce other cleistothecia, at times a large aggregate of them. While on rice and other natural mediums (carrot, potato) the wall of the cleistothecium is strong and thick, on barley and in maltose agar the wall is thin, smooth, without emitting secondary ascogenous hyphae. The ascogon produces an extremely fine net of ascogenous hyphae, which in turn, produce a very great number of asci with eight spores. The ends of these ascogenous hyphae become swollen, similar in appearance to a baseball bat, then they break up into eight spores. The ascus wall is very thin and evanescent, then becomes a slimy mass which keeps the spores together for awhile. Later on, the inner structure of the cleistothecium disappears completely and hundreds of spores fill its cavity. Through a break in the cleistothecium wall the ascospores are finally discharged.

F. Discussion

In a recent paper (4) an the critical survey of the present stand of production of perfect organs of fructification in dermatophytes I underscored two facts.

One was that in the long history of endeavors, from Matruchot and Dassonville to Nannizzi, to force conidial forms of dermatophytes as we know them at present, to produce perfect organs of fructification, throughout failed except for the apparent success of Nannizzi (10) in Sabourand's form-genus, *Trichophyton*. However, Nannizzi's results have never been confirmed up to date. Nannizzi attributed his success to the special technic he introduced, i.e., to culture dermatophytes on organic human or animal material, such as feathers, leather, human hair, etc. Ciferri and Redaelli, using the same technic, failed to elicit perfect organs of fructification in dermatophytes. In 1927, NANNIZZI introduced another technic and culture medium, i.e., forest soil mixed with small pieces of old leather and feathers of fowl. On this medium he apparently elicited ascus formation in the "species" *Achorion gypseum* BODIN, 1907. The pycnidia, according to his drawings, were identical with those found by him in *Tr. radiolatum* SAB., and in *Tr. equinum* MATRUCHOT.

The other fact was, that CHALMERS & MARSHALL (1914) (6) were the very first who in Khartoum (Sudan) found a new fungus, common in that geographical area, in tinea capitis, a *Trichophyton endothrix*, the *Tr. currii* CHALMERS & MARSHALL, which produced perfect organs of fructification spontaneously in the primary culture.

In my first chance observation I realized that the bacterial growth, the B. *weidmaniensis,* along with the colony of *M. Audouinii,* had a vital influence on the development of the conidial (development of macroconidia) as well as of the perithecial stage (development of cleistothecia) of this fungus.

In the history of medical mycology this was the first time observed that a bacterial symbiont exerted such a biologic effect upon a dermatophyte which led to the formation of perfect organs of fructification in a fungus in which only the conidial stage was known.

It is also noteworthy that the identical perfect organ of fructification in SABOURAUD'S form-genus *Microsporon* (pro parte) has been found in two entirely different ways. First, it was elicited by means of an incidentally found symbiont, the *B. weidmaniensis.* Secondly, it was found directly in the primary culture of *Microsporon*-hairs of tinea capitis, spontaneously. These findings mutually confirm each other.

These facts also break the old belief that fungi with perfect organs of fructification could not appear in a fungus infection of the hair and/or skin.

It also breaks with the already almost axiomatic belief that there are only one or two avenues of approach to elicit the perfect organs of fructification: 1) either to use human and/or animal keratinous material (hair, feathers, leather, hoofs, etc.) or 2) let the fungi (dermatophytes) grow in their imperfect stage in the "good earth": forest or garden soil.

Only within the narrow limits of dermatomycology could this concept arise. Stepping into the wider field of general mycology one can visualize at once that there is a kaleidoscopic variety of conditions which may cause imperfect fungi to produce higher organs of fructification.

DADE (8) gave many examples in this respect. I consider these facts so important in the interest of broader knowledge and for the sake of future research in this field that I will have to quote these passages.

> "*In nature, fungi grow in association with other fungi*, with bacteria, actinomycetes, protozoa, and other organisms. Their isolation in pure culture is the first fundamentally wrong treatment, but for obvious reasons we must often apply it. All the "undesired" organisms are discarded, yet one or more of them *may be an essential symbiont or commensal*. This phenomenon is probably much more common than may be supposed although many cases have been reported and others are known. The following are examples:
>
> "Heald and Pool (1909) found *that Melanospora zamiae would produce perithecia only when grown with Nigrospora* oryzae, *Gibberella fujikuroi*, or less successfully, with Fusarium culmorum and mention that Sordaria longicaudata could form perithecia only after Pilobolus had grown on the horse dung used as a medium. Before that date Molliard (1903) had shown that Ascobolus is similarly dependent on the presence of bacteria. McCormick (1925) succeeded in getting perithecia of Thielavia basicola in mixed culture with Thielaviopsis basicola and some other fungi, or on extracts or killed cultures of them. Mason (1933) observed that Acremoniella atra and A. verrucosa would not grow at all unless in company with a Cephalosporium sp., and also that Melanospora damnosa would produce perithecia only when grown with Thyridaria rubronotata. Dade (1937) *found that Thielavia setosa required the presence of Aspergillus flavus (or other moulds), or killed cultures, for the production of perithecia*. Petch (1925) had a similar experience with Melanospora parasitica and its associate Cephalosporium longisporum, and we have found that the latter stimulates other Melanospora spp. to some extent, as do other Cephalosporium spp. naturally associated with M. zamiae. Some years ago we received a culture of Ceratocystis sp. "contaminated" by Gonatobotryum fuscum. When they were separated the Ceratocystis remained obstinately sterile; when they were reunited, large crops of perithecia were formed. G. fuscum was then introduced to two South American isolates of another Ceratocystis sp. which had never formed perithecia but had been tentatively identified by their conidia. Dense stands of perithecia were then produced, and the earlier provisional identification was shown to be incorrect. *More recently a new and remarkable member of the Mucorales, not yet described, has formed its sporangia only in the presence of bacteria derived from the natural substratum, leaves, and found in the first isolation plates.*"

My findings have also definitely broken that so often repeated but never proven assumption that all the common dermatophytes, — the four form-genera of SABOURAUD: *Trichophyton*, *Microsporon*, *Epidermophyton*, *Achorion*, — must necessarily belong to the Gymnoascaceae. NANNIZZI's idea was that since aleuries, macroconidia, racquet mycelia, etc., occur in dermatophytes and in a number of species of Gymnoascaceae (*Myxotrichum*, *Arachniotus*, etc.), thus, the dermatophytes must consequently belong to this genus.

Conidial organs of reproduction possess an extremely wide variety of forms. With my discovery, these analogies between a number of saprophytes and the dermatophytes lost all of their taxonomic values. It turned out that the commonest dermatophyte of tinea capitis in the greater part of the world, belongs to an entirely different order and different genus than NANNIZZI presupposed. With this knowledge at hand, the conidial appearance of an imperfect fungus becomes, from the standpoint of taxonomy, irrelevant, since we know that a large group of the most different conidial forms of imperfect fungi may belong to one single perfect form. Only a few examples are given here:

Venturia DE NOT & CES. an ascomycete, has as its conidial stages

Fusicladium and *Spilocaea*. *Glomerella* SCHRENK & SPAULD., another ascomycete, has two imperfect fungi as its conidial stage: *Colletotrichum* and *Gloeosporium*. *Ceratocystis* HALSTEAD & FAIRCHILD has quite a number of imperfect fungi as its conidial stage, i.e., *Thielaviopsis*, *Chalaropsis*, *Graphium*, *Scopularia*, *Haplographium*, *Cephalosporium*, *Cladosporium*, as well as two of them simultaneously.

NANNIZZI was apparently successful with his special technic to elicit perfect organs of fructification (pycnidia, the lowest form of a higher organ of fructification in ascomycetes) only in the form-genus *Trichophyton*; in the form-genus *Microsporon* (*M. Audouinii*, *M. lanosum*), he admittedly failed (10). Later on, he reported exactly the same pycnidia (according to his drawings) in *M. gypseum* BODIN, 1907 (11). Based upon investigations in progress at my laboratory which will soon be published, I earnestly doubt whether the pycnidia found by NANNIZZI in *M. gypseum* BODIN, 1907, has belonged as a higher organ of fructification, to this fungus.

The new genus **Veronaia,** thus, encompasses besides the new type species *Veronaia Castellanii*, the old *M. Audouinii* GRUBY, *M. felineum* FOX & BLAXALL. The latter have sufficient amount of micromorphologic, macroscopic (the structure of the giant colony, its color, etc.) characteristics, including different degrees of pathogenicity, to be conserved as valid species.

My discovery shall also put an end to the aimless arrangement and rearrangement in the taxonomy of dermatophytes. In a forthcoming subsequent publication, I will be able to show that other ,,*Microsporon*'' fungi not included in the new genus *Veronaia*, belong to an entirely different genus which likewise, is not that of Gymnoascaceae! In 1948 while discussing the newer trend of classification in dermatophytes (3) I had already expressed my conviction that even when we would have the perfect organs of fructification of all the common dermatophytes, we would have to retain a dual system of taxonomy: SABOURAUD'S system of four form-genera, *) if for none other reason than to teach medical mycology on an undergraduate level, and a true taxonomy of the dermatophytes based upon the natural system of perfect organs of fructification. As one can easily see, the natural system of classification will, and at present already does completely disrupt any and all innumerable, artificial classifications of dermatophytes suggested in the last three or four decades. It is obvious that teaching medical mycology on the undergraduate level with an absolute lack of knowledge of botanical and/or general mycological background on the parts of the students would create only complete confusion in their minds.

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*) CIFERRI retained *Achorion* (REM.) CIF., as a subgenus in his recent classification, p. 417, Manuale di Micologia Medica, Vol. II, Casa Editrice Renzo Cortina, Pavia, 1960.

In SABOURAUD'S system, the student sees the relationship of fungi with respect to hair and skin; can see and grasp what an endothrix or an ectothrix fungus is and WHY it is; can see and understand that *Epidermophyton* fungi do not attack the hair. For the clinician this is the only vital matter. Whatever the position of a *Microsporon* fungus in the natural system may be, they attack the hair in an identical fashion: such as an ectothrix forming a spore sheath in mosaic pattern around the hair shaft. This, of course, is one type of unity. The natural system, on the contrary, would show at the onset, that the causative fungi in tinea microsporina, for instance, belong at least to two different unrelated genera.

Finally, a few words should be said concerning the new nomenclature. The new genus *Veronaia* is established according to the principles and rules of the International Code of Botanical Nomenclature (1956). The genus is characterized by the type species, in this instance, *Veronaia Castellanii* BENEDEK, 1960. This taxonomical approach is completely different from all the many "classifications" published the last three decades encompassing all the dermatophytes, based upon one or two common or different morphological features such as aleuries, macroconidia, etc. This newest approach is based upon the natural system of classification, i.e., the perfect organ of fructification. It does not intend to swallow up at once all four form-genera of dermatophytes. As the discovery of more and more perfect organs of fructification progresses, the species of the four old form-genera will be broken up and the individual imperfect, conidial forms of the dermatophytes will be assigned as conidial stages to this or that perfect form. Whether the present so-called species of any of the existing classifications of dermatophytes should be preserved or eliminated, will be a matter of further future discussion.

Note: — In 1941, strains of *B. weidmaniensis* BENEDEK, 1938 and of the two strains of *Microsporon* with the spontaneous production of perfect organs of fructification were deposited at the American Type Culture Collection. Recent correspondence (1959) with the ATCC revealed that these strains are no longer present in the Collection, thus, are lost to posterity. However, it is hoped that a concerted search and close observation may turn them up again at sometime, somewhere.

SUMMARY

The elicitation of perfect organs of fructification in the form-genus *Microsporon* (pro parte) by means of an incidentally found bacterial symbiont, the *B. weidmaniensis* BENEDEK, 1938 is described.

Furthermore, it is reported on two strains of *Microsporon* which spontaneously yielded identical perfect organs of fructification in the primary culture without any stimulation.

A new genus is established: *Veronaia* BENEDEK (Ascomycetes, Perisporiaceae, Aspergillales) which encompasses at present, 1) the type species: *Veronaia Castellanii*, BENEDEK, 1960; 2) *Veronaia* (*Microsporon*) *Audouinii* GRUBY and 3) *Veronaia* (*Microsporon*) *felinea* FOX & BLAX.

The genus *Veronaia* is cleistocarp and homothallic.

The Latin and English diagnoses of the type species (genus) are given.

The historical importance of this discovery and its impact on future search for the perfect organs of fructification in dermatophytes is discussed.

ZUSAMMENFASSUNG

Die Auslösung einer typischen Schlauchfruktifikation in der Form-Gattung *Microsporon* (pro parte) mittels eines zufällig gefundenen bakteriellen Symbionten *B. weidmaniensis* BENEDEK, 1938 wird beschrieben.

Ferner wird über zwei Stämme von *Microsporon* berichtet, die in der Primärkultur gleiche Schlauchfruktifikation spontan, ohne irgendeinen Reiz, geliefert haben.

Eine neue Gattung im natürlichen System wird aufgestellt: *Veronaia* BENEDEK (Ascomycetes, Perisporiaceae, Aspergillales). Sie umfasst zur Zeit 1) die Typen-species: *Veronaia Castellanii* BENEDEK, 1960; 2) *Veronaia* (*Microsporon*) *Audouinii* GRUBY; 3) *Veronaia* (*Microsporon*) *felinea* FOX & BLAX.

Die Gattung *Veronaia* ist cleistocarp und homothallisch.

Die lateinische und englische Diagnose der Typen-species (Gattung) ist gegeben.

Die geschichtliche Bedeutung dieser Entdeckung und ihr Einfluss auf zukünftige Untersuchungen betreffs der Schlauchfruktifikation in den Dermatophyten wird erörtert.

RÉSUMÉ

L'auteur reporte sur la découverte de la vraie forme de réproduction dans le forme-genre *Microsporon* (pro parte) de SABOURAUD par le moyen d'un symbionte bactérien, le *B. weidmaniensis* BENEDEK, 1938, découvert incidemment.

En outre, il reporte sur deux souches de *Microsporon* lesquelles ont produit la même forme parfaite de réproduction, spontanément dans la culture primaire sans aucune stimulation.

Un genre nouveau est établi: *Veronaia* BENEDEK, gen. nov. (Ascomycetes, Perisporiaceae, Aspergillales) en renfermant à présent (1) l'espèce-type: *Veronaia Castellanii*, BENEDEK, 1960; (2) *Veronaia* (*Microsporon*) *Audouinii* GRUBY et (3) *Veronaia* (*Microsporon*) *felinea* FOX et BLAX.

Le genre *Veronaia* est cleistocarpe et homothallique.

Le diagnostic latin et anglais est assigné à l'espèce-type (genre). L'importance historique de cette découverte et son influence sur les recherches futures ont été discutées.

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NUTRITIONAL REQUIREMENTS OF ARTHRODERMA TUBERCULATUM

by

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(24.IX.1960)

Recently a new species of *Arthroderma*, *A. tuberculatum*, was described by KUEHN (1960). This species, in common with certain other Gymnoascaceae, lost the ability to produce ascocarps after repeated transfer on YpSs and Sabouraud's agar media. Attempts to maintain stock cultures on other, defined, media failed because the fungus did not grow on such media. Therefore, a study of the nutritional requirements of this ascomycete was undertaken in shaken flasks to enable us to devise a defined medium which would support optimum vegetative growth. Future investigations employing such a medium in the form of a solid agar medium are planned to elucidate the physiological requirements for ascocarp formation in *A. tuberculatum*.

MATERIALS AND METHODS

The culture of *Arthroderma tuberculatum* used in this investigation was the type strain NRRL 2853 (Northern Utilization Research and Development Division, USDA, Peoria, Illinois). The experimental procedures were similar to those presented in detail by KUEHN & CROSBY (1959) and, briefly, were as follows. Cultures were grown in duplicate using 100 ml medium in 500 ml Erlenmeyer flasks which were incubated usually from 3 to 5 days on a rotary (Gump) shaker. Preliminary experiments showed that the fungus failed to grow at 37° C. Experimental flasks were inoculated with 1 % of 3-10 day old submerged growth brought up in inoculum flasks of Sabouraud's medium (4 % maltose, 1 % peptone). Following the desired period of incubation the mycelium was removed from the culture broth by centrifugation and the dry weight was used as an index of growth. The pH of the supernatant was recorded as the terminal pH.

As already mentioned, YpSs and Sabouraud's agar media supported ample growth of *A. tuberculatum*. Therefore, these media were employed in preliminary studies to determine the optimum initial pH and the rate of growth as a function of time. The constituents of these media are: YpSs - 1.5 % soluble starch, 0.4 % yeast extract, 0.1 % K_2HPO_4 and 0.05 % $MgSO_4.7H_2O$; Sabouraud's - 4 % maltose, 1 % peptone. Further experiments were performed to determine the availability of various nitrogen and carbon sources and requirements for growth factors, including certain vitamins, purines and pyrimidines.

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Results and Discussion

Preliminary experiments to determine the influence of initial pH of the medium upon growth of *A. tuberculatum* revealed that pH 7. 0-8.0 was the optimum initial pH range. Studies of rate of growth as a function of time in YpSs and Sabouraud's media showed that maximum dry weight was attained in each medium after about 80 hours of incubation. Furthermore, in Sabouraud's medium the dry weight reached 1,009 mg per 100 ml broth while in YpSs medium the maximum dry weight was only 283 mg per 100 ml.

In order to study carbon assimilation various carbon-containing compounds were used at a concentration of 4 % with 1 % peptone as the nitrogen source, with an initial pH of 7.0. Table I shows the results with the compounds listed in descending order of utilization. The amount of growth with succinic acid was about twice as great as with the next best carbon source in this experiment incubated for 72 hours.

TABLE I.

Assimilability of various carbon-containing compounds

Compound	Terminal pH	Mycelial weight mg/100 ml
succinic acid	7.8	2251
mannose	7.2	1378
itaconic acid	8.4	1297
glucose	7.7	1071
sucrose	8.6	1052
maltose	8.6	985
soluble starch	8.6	933
fructose	7.7	877
sorbitol	8.5	876
lactose	8.6	733
sodium acetate	8.0	664
sorbose	6.5	437
citric acid	7.6	423
controls	8.6	339
xylose	5.1	69

Various nitrogen-containing compounds were compared for their ability to support growth of *Arthroderma tuberculatum* in a medium containing 4 % succinic acid and 1 % of the nitrogen source, at pH 8.0. After 72 hours of incubation no substantial growth had occurred in any flask except those containing a complex nitrogenous source such as yeast extract, peptone or corn steep liquor. Several experiments were conducted using succinic acid and any one of a variety of nitrogen-containing compounds but in no instance did growth occur except when a complex source of nitrogen was used. The basal medium was supplemented with a variety of vitamins, organic bases and inorganic salts, nevertheless, growth still did not result when defined nitrogen sources were supplied. Table II lists the nitrogen compounds which were tested for utilization, at a 1 % level, with succinic acid as the carbon source.

TABLE II.

Nitrogen containing compounds tested for assimilability with succinic acid as the carbon source.

yeast extract
peptone
glycine
KNO_3
$NaNO_3$
lysine
$(NH_4)_2SO_4$
urea
ammonium acetate
corn steep liquor
NH_4NO_3
dl alpha alanine
dl aspartic acid
l glutamic acid
dl asparagine
dl isoleucine
dl methionine
dl valine
l cystine
l arginine
l proline
l tyrosine
dl phenylalanine
dl tryptophan
dl threonine
$(NH_4)_2HPO_4$
$Ca(NO_3)_2$
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$

Since peptone supported growth, an attempt was made to devise a basal medium which contained most of the components found in peptone. The composition of this basal medium is given in Table III. However, such a medium did not support growth of *A. tuberculatum* with succinic acid as the carbon source. This medium did support growth if glucose was the carbon source, although growth was not as great as with peptone. Further investigations made it clear that although succinic acid was able to support growth with a complex nitrogen source such as peptone or yeast extract, the fungus was not able to utilize succinic acid as a source of carbon when a single, defined nitrogen source was supplied. The reason for this seeming paradox has not become apparent to us.

Since glucose had been demonstrated to be one of the better sources of carbon (Table I) the assimilability of nitrogen compounds was re-investigated using glucose as the carbon source. Table IV illustrates the results of such an experiment incubated 89 hours which shows that no single defined nitrogen source was as good as one of the complex sources.

Several of the better, single, defined sources of nitrogen were tested at various concentrations, including 1,2 and 3 %. It was

TABLE III.

Composition of a basal medium designed to supply most of the compounds present in a natural peptone

Compound	Concentration mg per cent
glycine	20
alanine	10
glutamic acid	10
aspartic acid	10
asparagine	10
urea	10
arginine	10
NH_4NO_3	10
valine	1
lysine	1
isoleucine	1
tyrosine	1
phenylalanine	1
thiamine	0.1
biotin	0.1
pyridoxine	0.1
riboflavin	0.1
niacin	0.1
calcium pantothenate	0.1
xanthine	1
guanine	1
adenine	1
uracil	1
KH_2PO_4	100
$MgSO_4.7H_2O$	50

TABLE IV.

Growth of Arthroderma tuberculatum with various nitrogen containing compounds and with glucose as the source of carbon

Compound	Terminal pH	Mycelial weight mg/100 ml
corn steep liquor	6.6	1057
peptone	8.0	886
yeast extract	8.1	743
NH_4NO_3	6.0	539
l glutamic acid	6.8	438
dl aspartic acid	7.4	423
dl alpha alanine	7.3	371
lysine	5.5	349
KNO_3	5.8	348
$NaNO_3$	5.9	299
$(NH_4)_2SO_4$	6.0	271
ammonium acetate	6.2	238
glycine	7.8	233
urea	8.0	189
controls	5.8	186

found that glutamic acid at a 2 % level supported about as much growth as 1 % peptone, and with 3 or 4 % glutamic acid the growth reached over 1000 mg per 100 ml broth. None of the other defined nitrogen compounds gave significantly more growth at concentrations above 1 %. Mono sodium glutamate was found to be equivalent to glutamic acid in supporting growth of *Arthroderma tuberculatum.*

A survey was made of various vitamins and organic bases to determine whether addition of such compounds might stimulate growth in a glucose-glutamic acid medium. However, such supplements did not increase growth.

TABLE V.

Influence of various concentrations of glucose and glutamate upon growth of Arthroderma tuberculatum.

		per cent mono sodium glutamate						
		0.0	1.0	2.0	3.0	4.0	5.0	6.0
per cent glucose	0.0	98 1)	191	309	410	630	603	665
	1.0	45	961	736	713	924	1102	1390
	2.0	61	680	719	780	1183	1323	1457
	3.0	52	642	977	1077	1352	1585	1656
	4.0	43	840	1026	1712	1334	1930	2388
	5.0	51	1153	1215	1503	1744	1834	2140
	6.0	54	1037	1107	1775	1992	2229	1952

1) denotes mg mycelium per 100 ml medium.

Experiments were performed to determine the optimum levels of glucose and glutamate. Table V shows the results of such an experiment incubated for 5 days in which the only components of the medium were glucose, mono sodium glutamate, 0.1 % K_2HPO_4 and 0.05 % $MgSO_4.7H_2O$. Maximum cell yields were attained with the higher levels of glucose and glutamate, with the highest growth coming with 6 % glutamate and 4 % glucose.

The optimum synthetic medium developed in these studies was used in one final experiment to find the incubation time required to permit maximum growth. The medium consisted of 6 % mono sodium glutamate, 4 % glucose, 0.1 % K_2HPO_4 and 0.05 % $MgSO_4.7H_2O$ at pH 7.5 Results are shown in Table VI and reveal that maximum growth is produced in about $4\frac{1}{2}$ days.

Kuehn (1960) isolated two strains of *Arthroderma tuberculatum,* one from an owl pellet, the other from the feather of a robin. The particular strain used in this nutritional study was the one which was isolated from a feather, which represented a keratinophilic habitat. Since keratin is composed of about 13 % glutamic acid, it is not surprising that glutamic acid, used as a sole source of nitrogen, was able to support growth of *Arthroderma tuberculatum.* It will be interesting to study the influence of this medium on ascocarp formation.

TABLE VI.

Rate of growth of Arthroderma tuberculatum in a defined medium as a function of time.

Hours of Incubation	Terminal pH	Mycelial weight mg/100 ml medium
66	6.9	1347
90	8.0	1622
114	8.3	2853
138	8.3	1556
162	8.3	1631
234	8.4	1722
258	8.5	1724
282	8.5	1595

SUMMARY

Certain nutritional requirements of one strain of *Arthroderma tuberculatum* were established in shaken flask culture. Growth was measured on a dry weight basis. The optimum medium for growth consisted of 6 % mono sodium glutamate (or glutamic acid), 4 % glucose, 0.1 % K_2HPO_4 and 0.05 % $MgSO_4.7H_2O$, with an initial pH range of 7.0-8.0. Maximum growth was attained in this medium after about 4½ days incubation at 28 °C on a rotary shaker.

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FUNGI FROM ALBERTA RODENTS

by

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(24.IX.1960)

In 1947, Dowding reported on the results of a search for pathogenic fungi in Alberta rodents. She found *Emmonsia parva* (Emmons & Ashb.) Cif. & Montemart.[1]) in fourteen out of 275 animals. Since that time, workers from this University have continued the search during most summers. With the exception of a note on *Endogone* (Dowding, 1955) none of the work subsequent to 1946 has yet been reported. This paper summarizes the findings of interest from the rodent surveys for the period 1946 to 1959.

Materials and Methods

Animals.

Most of the animals were trapped or shot during independent entomological and zoological surveys. Collections were made in the spring and summer from all parts of Alberta which are accessible by road. That is, most of the southern half of the province plus the Peace River and Fort Vermillion areas. A few animals collected specifically for fungus studies were trapped in the Edmonton area by Dr. A. Bakerspigel and by the author.

The animals examined included representatives of most of the small mammals indigenous to Alberta, including rabbits and carnivores as well as rodents, but by far the largest number of collections were deer mice (*Peromyscus maniculatus*), ground squirrels (*Citellus richardsoni*), meadow mice (*Microtus pennsylvanicus*), house mice (*Mus musculus*) and red-backed mice (*Clethrionomys gapperi*).

Autopsy and Examination.

Most of the autopsies were performed in the field using specially equipped trucks as mobile laboratories (Govt. of Alberta, 1948).

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[1]) ≡ *Haplosporangium parvum* Emmons & Ashburn 1942.

The animals were soaked in lysol before being opened and four separate sets of boiled scissors and forceps were used for each animal. Procedures for the detection of fungi consisted of: - (a) Gross examination - a visual examination of the internal organs for any abnormalities. Mature lesions due to the presence of *E. parva* have a distinctive appearance to the trained eye, and in some years only those animals having such lesions were subjected to further examination. (b) Microscopic examination - a study of hematoxylin and eosin stained sections of lobes of lungs fixed in formalin. In 1946 all animals were examined in this way. In 1951 about 10% of the lungs were sectioned. In other years, sections were prepared only from those animals which showed gross lesions, or from which interesting fungi were recovered in culture. (c) Cultures - made by cutting off a piece of the selected organ and mashing it around on the surface of an agar slant in a screw-capped bottle. Lung samples consisted of the lower 1/2 to 2/3 of one lobe. Spleen samples consisted of about 1/3 of the spleen. The bottles were shipped by express to the Provincial Laboratory at the end of each day's operations. During 1949 and part of 1950 petri dish cultures were used rather than slants. Various culture media were used up to 1950, including Sabouraud's agar, Littman's OSCV agar, blood agar and brain-heart infusion agar. From 1955 the standard medium used was a modified Sabouraud's agar with yeast extract and antibiotics added (CARMICHAEL & KRAUS, 1959). This medium is now available from Baltimore Biological Laboratory as Phytone Yeast Agar.[1]) In 1949 cultures were made from the lungs, liver and spleen. In other years, only the lungs or the lungs and spleen were cultured. All cultures were incubated at room temperature and examined at frequent intervals for fungus growth.

RESULTS

A. Emmonsia parva infection

Much of the survey work has been focused on a search for *Emmonsia parva*. After the initial findings of 5.1% infection in 1946 and 3.3% in 1947, a very low rate of infection was found in subsequent years (Table I). We can offer no explanation for the apparent decline and there are no other published, long-term studies with which these findings may be compared. The survey is being continued and it is possible that a cyclical trend may eventually be disclosed.

B. Mold Flora of Rodent Lungs.

Although the survey has, in the past, been aimed primarily at detecting *E. parva*, one of the most interesting findings has been

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[1]) Baltimore Biological Laboratory, Baltimore 16, Maryland, U.S.A.

TABLE I.

Findings of Emmonsia parva by Years

Year	No. of Animals Examined	No. with *E. parva*	Methods	Investigators
1946	275	14	Sections - lungs only Cultures - lungs only	DOWDING
1947	121	4	Gross examination Cultures - lungs only	DOWDING
1948	77	0	Cultures - lungs only	DOWDING
1949	62	0	Cultures - lungs, liver, spleen	DOWDING & CARMICHAEL
1950	119	1	Cultures - lungs, spleen	DOWDING & CARMICHAEL
1951	702	1	Gross examination [1]	DOWDING
1952	1,367	1	Gross examination	DOWDING & BAKERSPIGEL
1953	141	1	Gross examination	DOWDING & BAKERSPIGEL
1954	0			
1955	23	0	Cultures - lungs and spleen	CARMICHAEL
1956-58	0			
1959	106	0	Cultures - lungs and spleen	CARMICHAEL

[1] Organs showing suspicious lesions were sectioned and examined microscopically for chlamydospores of *E. parva*.

TABLE II.

Frequency of Isolation of certain Fungi from Rodent Lungs and Spleens

Growth in Culture	1950 series 22 animals		1959 series 106 animals	
	Lungs	Spleen	Lungs	Spleen
No growth	0	14	16	41
Penicillium spp.	18	0	43	16
Beauveria bassiana (BALS.) VUILL.	11	0	6	0
Aspergillus spp.	6	0	12	4
Scopulariopsis brevicaule (SACC.) BAIN.	4	1	3	1
Cladosporium spp.	11	5	15	16
Chrysosporium pannorum (LK.) HUGHES	[1]	[1]	4	2
Yeasts	0	1	0	9

[1] Probably present, but not identified in 1950.

that the lungs of rodents have a characteristic mold flora. This gradually became apparent during the first few years of the survey, but was obscured by the high rate of contamination in the cultures made in the field. The most decisive evidence came from a series of 22 animals which were trapped in the vicinity of Edmonton in 1950 and autopsied at the Provincial Laboratory with the utmost care to avoid contamination. Table II shows the number of animals from which certain fungi were isolated from the lungs and from the spleen in the special 1950 series and also in the regular 1959 field cultures.

The fact that no growth occurred in two-thirds of the spleen cultures in 1950 is good evidence that the rate of contamination was low. Such being the case, the obvious conclusion is that most of the fungi isolated in the cultures of lungs actually came from the lungs. *Beauveria bassiana* and species of *Penicillium* and *Aspergillus* were the predominant and distinctive organisms. *Cladosporium spp.* and *Scopulariopsis brevicaule* were also common, but not restricted to the lungs. Further evidence that these fungi were not chance contaminants is provided by the large number of colonies which appeared in many of the lung cultures. In seven of the 22 cultures there were 10 to 35 colonies of *Beauveria* or *Penicillium* on the plate, and in three lung cultures the colonies of *Beauveria* (2 animals) or *Penicillium* (1 animal) were too numerous to count. None of the spleen cultures had more than 6 colonies and the average for the 8 cultures which showed growth was only 2.5 colonies per plate as compared to an average of more than 13 colonies per plate for all the lung cultures.

As a check that the fungi isolated from lungs were not air contaminants, a series of 25 plates was exposed to the air in the room where the animals were autopsied, in other parts of the laboratory, and outside the laboratory. The flora on these plates was surprisingly uniform for the various locations and quite different from the rodent lung flora, with the exception of the high incidence of *Cladosporium* in both. The total numbers of colonies of the most common organisms on the 25 exposed plates were: *Cladosporium*, 73; Sphaeropsidales, 17; yeasts, 9; *Alternaria*, 6; *Scopulariopsis brevicaule*, 5; *Aspergillus*, 4; and *Papularia*, 4. There was only one colony of *Penicillium* and none of *Beauveria*.

The 1959 series of 106 field cultures (Table II) generally confirms the findings in the 1950 series of laboratory cultures.

Although sections of more than 300 rodent lungs have been examined during the course of this survey, hyphae or yeast cells were never seen in the lung tissue; neither were spores of *Penicillium* or *Beauveria*. However, the small spores of these latter fungi would not likely be detected, even if present in large numbers, unless a special search were conducted using an effective differential stain.

Rodents live in close contact with the soil and with the plant debris on the surface. It seems reasonable to suppose that they are exposed to large numbers of fungus spores. The results of our cultures suggest that some of the smaller mold spores, such as those of some species of *Penicillium* and *Aspergillus*, not only get past the respiratory filter system and reach the lower lungs, but that they survive in that location for some time. The presence of *Beauveria bassiana* is not surprising when one remembers that insects comprise a substantial part of the diet of most rodents.

C. Trichophyton from Rodent Organs

In 1949, Trichophytons were isolated from the livers of two animals which appeared grossly normal at autopsy. The first was a deer mouse (*Peromyscus maniculatus*) trapped near Taber in May. On the plates streaked with the liver, two colonies grew which were identified as *Trichophyton sp.*, probably *T. mentagrophytes*, on the basis of the morphology of the microconidia and the colonies. Unfortunately definite identification was not obtained before the cultures were discarded. The second animal was a house mouse (*Mus musculus*) trapped at the University Farm in Edmonton in July. On the plates streaked with the liver nine colonies grew which were entirely typical of the granular type of *T. mentagrophytes*. Microconidia, macroconidia and spirals were present in abundance.

During the 1959 survey Trichophytons were isolated from four animals -
S-653 lung, *Peromyscus maniculatus*, Slave Lake, July - two colonies; S-654 spleen, *Peromyscus maniculatus*, Ft. Vermillion, August - one colony; spleen, *Peromyscus maniculatus*, Smoky Lake, July - one colony; S-669 lung, *Citellus richardsoni*, Bittern Lake, May - one colony. The first three were typical *T. mentagrophytes*. The last one (S-669) was an undescribed species of *Trichophyton* which has also been isolated several times from local soil.

The isolation of *T. mentagrophytes* from the internal organs of wild animals has also been reported by EMMONS (1950). This finding provokes the question; how did the inoculum get there? There are at least four possibilities: 1. systemic infection or local internal infection, 2. stray spores entering the general circulation from a skin infection and surviving in the internal organs, 3. contamination from a skin infection during autopsy, or 4. inhaled spores, in the case of the lungs. Since there were no grossly visible lesions in any of the animals, the first possibility seems unlikely. The second possibility gains support from the work of GEORG (1954) who isolated *T. mentagrophytes* from the internal organs of two dogs with experimental skin infections. The third possibility cannot be ruled out, but should be a rare occurrence in this series where the first procedure at autopsy was to soak the entire animal in disinfectant. The last possibility is supported by the finding that rodent lungs normally harbour certain molds, presumably as inhaled spores. It is perhaps significant that the soil *Trichophyton* (S-669) was isolated from the lungs rather than from the liver or spleen.

Lurie & Borok (1955) demonstrated the presence of *T. mentagrophytes* in soil samples from two bat infested caves in Transvaal. Later, Lurie & Way (1957) exposed various animals to dust in one of the caves, held them for six to fifteen days, and then examined their lungs. Cultures of lung fragments did not yield *T. mentagrophytes*, but this fungus was recovered from the livers or spleens of some (number not stated) of 252 (?) mice inoculated intraperitoneally with macerated lung tissue. The authors concluded that the source of the *Trichophyton* was the atmosphere of the cave and that the organism must have multiplied in the inoculated mice. It now appears that *T. mentagrophytes* may have been present in the mice before they were inoculated.

D. Phycomyces from Rodents.

Although the genus *Phycomyces* is well known, it is not commonly found in nature and has not been previously reported from an animal source. It is of interest, therefore, to record two isolations of *Phycomyces* from rodent lung cultures. S-666 from *Peromyscus maniculatus* trapped near Keg River, in August, 1959 and S-667 from *Peromyscus maniculatus* trapped near Fort Vermillion in August, 1959. Both towns are approximately 58° N. latitude. These strains were examined by Dr. C. W. Hesseltine and identified by him as *P. blakesleeanus* Burgeff, minus mating type. They were atypical in that the sporangiospores were mostly globose rather than ovoidal.

Since there were no visible abnormalities in the mouse lungs at autopsy, there is no way of knowing whether these strains came from the lung tissue or whether they were contaminants.

Summary

The occurrence, by years, of *E. parva* in Alberta rodents is presented in tabular form covering the period 1946 to 1959. The infection rate was 5.1.% in 1946, 3.3% in 1957 and less than 1% thereafter.

It was found that the lungs of Alberta rodents have a characteristic mold flora comprised chiefly of : *Penicillium spp.*, *Beauveria bassiana*, *Aspergillus spp.* and perhaps *Scopulariopsis brevicaule*, *Cladosporium spp.* and *Chrysosporium pannorum.*

Trichophyton mentagrophytes and another *Trichophyton* were isolated from the livers, spleens or lungs of six rodents.

Phycomyces blakesleeanus was isolated from the lungs of two rodents trapped in northern Alberta.

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GROWTH CHARACTERISTICS OF *DACTYLELLA DRECHSLERI* N. Sp., AN ADHESIVE-KNOBBED, NEMATODE-TRAPPING HYPHOMYCETE FROM FLORIDA[1]

by

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(with 2 figs.)

(15.XI.60)

The nematophagous fungi currently are being re-investigated, in various parts of the world, for possible utilization as controlling agents for nematode diseases of plants. With this purpose in mind, I have collected numerous fungi from citrus groves in Central Florida — microflorae which, for the most part, are referable to previously described species. The new fungus described hereafter was an exception, isolated from roots and soil collected from a mature grapefruit grove in an excellent state of vigor.

When portions of the collected soil or roots were implanted on corn meal agar and held at 75° F., the fungus grew relatively slowly, becoming macroscopically distinct, after five to eight days. Conidiophore production and sporulation was prolific; predacious habit, consisting of stalked adhesive knobs, was expressed regardless of the presence of nematodes in cultures.

In the earlier stages of this investigation the fungus was throught to be identical with *Dactylella ellipsospora* (PREUSS) GROVE because of similarities in conidium and adhesive knob shape, as well as growth habit. Yet, several consistent dissimilarities with the aforementioned species, the most important of which was the abundant production of triseptate spores in contrast to the usual quadriseptate spore formed by *D. ellipsospora*, determined the novel identity of the Florida fungus.

The original accounts by ZOPF (7) for his *Monosporidium repens* (believed by DRECHSLER (2) to be identical to his *D. ellipsospora*), and by BUBÁK (1) for his *Monacrosporium leporinum* (believed by both DRECHSLER (2, 3) and DUDDINGTON (4) to be identical to *D. ellipsospora*) were examined and their critical data compared

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[1]) Florida Agricultural Experiment Station Journal Series, No. 1200.

with that of the Florida form. Likewise, descriptions of *D. ellipsospora* by GROVE (5), SMITH (6), DRECHSLER (3), and DUDDINGTON (4) were evaluated. These accounts failed to include essential length ranges for certain morphological data characteristic of *D. drechsleri*.

Although both pure cultures of *D. drechsleri* and cultures infested with the nematode *Panagrellus redivivus* (LINN.) were studied, the morphology of the fungus was apparently the same in both, as indicated by visual comparisons and measurements. The fungus formed a fine mycelium, exhibiting frequent anastomosing (Fig. 1, ac). Occasionally in both pure and nematode-infested cultures, spiral mycelial ramifications were formed which seemed to be purely vegetative and wholly without nematode-trapping ability (Fig. 1, ah). I have also found these structures formed by a number of other species of nematophagous fungi. Erect septate conidiophores were formed which eventually bore solitary conidia (Fig. 1, ag, ai, and ak); 31 conidiophores from a nematode-infested culture 2—1/2 weeks old measured 145 μ (93—184 μ) long. Occasionally a hyphal branch on which a secondary conidium was produced was formed from an aging conidiophore (Fig. 1, o). Conidia from nematode-infested cultures did not differ appreciably from those formed in pure culture. These were predominantly triseptate, although a considerable number of biseptate conidia were also found. Quadriseptate conidia, so typical of *D. ellipsospora*, were always scarce. From a two-week-old pure culture of the fungus growing on corn meal agar, 30 biseptate conidia were found to be 35.8 μ (29.5—39.8 μ) long and 12.8 μ (10.7—14.5 μ) wide; 35 triseptate conidia were 39.9 μ (34.2—45.9 μ) long and 13.2 μ (11.2—14.5 μ) wide; and 8 quadriseptate conidia were 43.0 μ (36.5—48.7 μ) long and 13.2 μ (10.7—15.0 μ) wide. Some of the conidia measured are illustrated in Fig. 1, a-h, p-q, t-u, y-z, aa, ad-ae, and aj.

Measurements of conidia were also made from a similar aged culture, but to which nematodes had been added a week previously. Thirty biseptate conidia were 33.9 μ (29.5—41.7 μ) long and 12.7 μ (10.3—14.5 μ) wide; 35 triseptate conidia were 38.8 μ (32.8—44.0 μ) long and 13.1 μ (9.8—15.0 μ) wide; and 8 quadriseptate conidia measured 43.4 μ (40.7—47.3 μ) long and 13.0 μ (10.7—14.5 μ) wide. These are illustrated in Fig. 1, j-n, r, ab, af, and al. Germination of conidia usually occurred after they had fallen on the surface of the substrate, although occasionally conidia would germinate while still aerially borne on the conidiophore. The basal cell of the conidium usually germinated first, with the newly-formed hypha originating either at the basal tip (Fig. 1, v) or at the side (Fig. 1, w and x) of the cell. Numerous conidia were observed in which both the apical and basal cell had germinated (Fig. 1, i). Occasionally, triseptate conidia were observed with germination processes emanating from the apical, sub-basal, and basal cells.

The adhesive knob formed by *D. drechsleri* consists of a terminal subspherical to spherical cell borne on a unicellular stalk arising

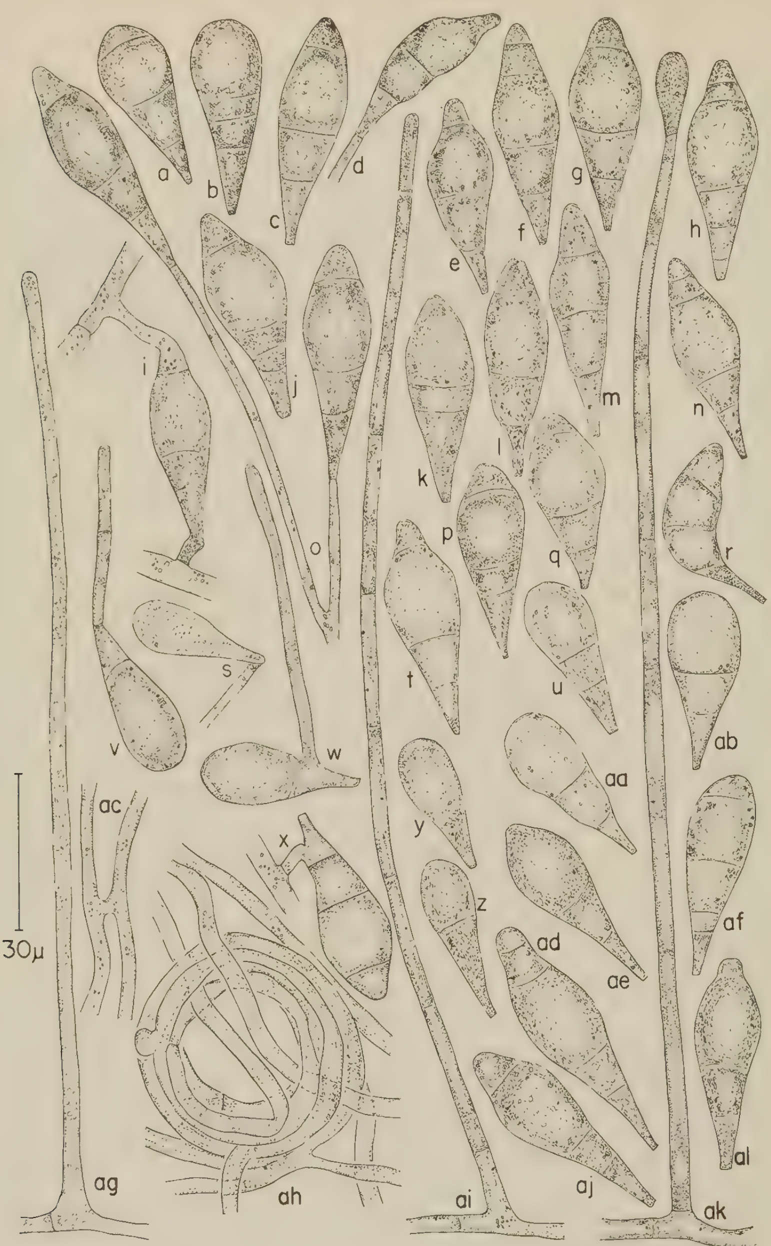

Fig. 1. *Dactylella drechsleri* n. sp. a-h, conidia from nematode-infested culture; i, conidium from pure culture showing germination of apical and basal cells; j-n, conidia from nematode-infested culture; o, terminal portion of a mature conidiophore from pure culture showing the lowermost primary conidium and the secondary conidium situated on a branch from the primary conidiophore; p-q, conidia from pure culture; r, curved conidium from nematode-infested culture; s, single-celled conidium from pure culture attached to conidiophore; t-u, conidia from pure culture; v-w, two-celled conidia from pure culture showing germination hyphae originating from basal cell; x, four-celled conidium from pure culture showing germination of basal cell; y-z, aa, conidia from pure culture; ab, conidium from nematode-infested culture; ac, mycelium from pure culture showing anastomosis; ad-ae, conidia from pure culture; af, conidium from nematode-infested culture; ag, conidiophore from pure culture; ah, spiral mycelial ramification from nematode-infested culture; ai, conidiophore from pure culture; aj, conidium from pure culture; ak, conidiophore bearing young conidium from nematode-infested culture; al, conidium from nematode-infested culture.

perpendicular to the parent mycelium (Fig. 2, b-f, i-j). Among 30 such structures produced in a nematode-infested culture, the terminal knob length was 9.0 μ (8.0—10.0 μ), the width 8.3 μ (7.2—9.6 μ). The stalk length (as measured between the spherical cell and the septate junction with the parent mycelium) was 7.9 μ (4.4—14.0 μ). The mycelium on which the stalked knobs were produced was 2.6 μ (1.6—3.6 μ) wide. Occasionally knobs were formed on hyphae originating from germinated conidia. Fig. 2, g shows one such conidium with 2 germination hyphae arising from the basal cell, one of which is a knob structure, and also a knob formed from the apical cell. Fig. 2, l shows a conidium of which the apical cell has germinated forming a separate hypha bearing 3 predacious knobs.

The operation of these adhesive trapping devices occurs as already described by Drechsler (2, 3). The victim upon becoming inseparably affixed to the adhesive knob, struggles violently. After death has occurred, or possibly slightly earlier, a finger-like process from the knob penetrates the integument of the nematode and forms a circular structure (Fig. 2, a and k) from which assimilative or trophic hyphae emanate (Fig. 2, h and m) growing somewhat parallel to the axis of the body of the victim. In a relatively short time the contents of the nematode body have been consumed by the fungus, which appears as a tightly packed bundle of mycelial strands within the nematode integument. When all available food within the nematode body has been utilized by the fungus, hyphae penetrate the nematode integument and enter the agar substrate.

Dactylella drechsleri appears sufficiently distinct from published descriptions of *D. ellipsospora* to justify its identity as a new species. Confirmation of this conclusion was furnished through comparison of the new species with a culture identified as *D. ellipsospora*. Further proof of the uniqueness of the Florida form was obtained by culturing it on different substrates, at varying pH, and at several temperatures.

Petri plates containing Difco potato dextrose agar (PDA), corn meal agar (CMA), and plain water agar (WA) at a 1.7 % concentration were inoculated with *D. drechsleri* and stored at temperatures of 65°, 70°, 75° and 80° F. There were two replicate plates per treatment. After 3 weeks, various measurements and observations were made of the fungus in each plate.

The results of this test as presented in Table I clearly show corn meal agar to be the most suitable substrate tested and 70° to 75° F. to be the best temperature range for optimum growth of the fungus. In this environment the fungus made a maximum lateral growth of 86 mm, and produced the largest and greatest number of conidia. An interesting corollary was the production of the highest percentage of triseptate conidia and the extreme paucity of quadriseptate conidia under these conditions which appeared optimum for fungus growth. The general failure of the fungus to form predacious

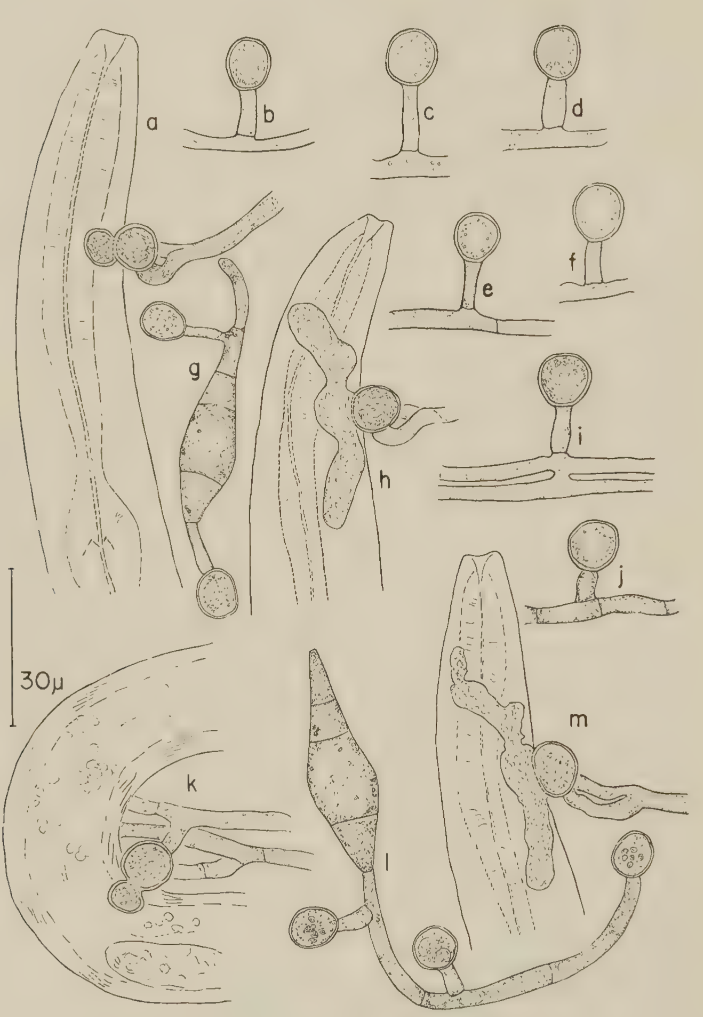

Fig. 2. *Dactylella drechsleri* n. sp. a, female *Panagrellus redivivus* trapped by an adhesive knob that has penetrated the cervical region of the nematode forming a globose haustorium-like body; b, adhesive knob from nematode-infested culture; c, adhesive knob from pure culture; d, adhesive knob from infested culture; e, adhesive knob from pure culture; f, knob from infested culture; g, conidium from infested culture showing germinated apical cell bearing stalked adhesive knob and basal cell with adhesive knob and finger-like hyphal process; h, parasitized nematode referred to in (a) above but 2-1/2 hours afterwards — note formation of trophic or assimilative hyphae within the victim; i, adhesive knob in infested culture formed from anastomosed mycelium; j, knob from infested culture; k, female *P. redivivus* trapped near mid-region of body; l, conidium from infested culture with germinated basal cell forming septate mycelial branch bearing the terminal and 2 lateral adhesive knobs; m, parasitized nematode referred to in (a) and (h) above, but 4-1/4 hours after (a).

TABLE I.

Growth characteristics of D. drechsleri in pure culture as affected by temperature and type of substrate.

Temperature	65° F.			70° F.			75° F.			80° F.		
Substrate	PDA	WA	CMA	PDA	WA	CMA	PDA	WA	CMA	PDA	WA	CMA
Width of growth zone in mm 1)	15	25	66	18	13	82	21	22	86	— 2)	— 3)	66
Relative degree of sporulation 1)	0 4)	++	++	0	++	+++	0	+	+++	—	0	0
Average conidium length in μ	—	36.2	37.6	—	37.3	43.5	—	34.6	36.9	—	—	—
Average conidium width in μ	—	10.1	13.0	—	11.4	13.9	—	9.9	12.5	—	—	—
Average knob length in μ	10.2 5)	10.6	9.5	— 6)	—	—	—	10.5	—	—	—	—
Average knob width in μ	10.0	9.8	8.5	—	—	—	—	8.4	—	—	—	—
Average stalk length in μ	66.6	37.0	10.2	—	—	—	—	10.0	—	—	—	—
% conidia-1 septum	—	36 7)	4	—	17	4	—	27	4	—	—	—
% conidia-2 septa	—	16	21	—	26	21	—	23	4	—	—	—
% conidia-3 septa	—	48	71	—	57	75	—	50	88	—	—	—
% conidia-4 septa	—	0	4	—	0	0	—	0	4	—	—	-
Presence of spiral mycelial ramifications	no	yes	yes	no	yes	yes	no	yes	no	—	no	yes

1) average measurements of 2 replicates

2) no growth

3) only sparse growth

4) Code:
0 = none
+ = light
++ = moderate
+++ = heavy

5) average measurements of 2 replicate knobs

6) predacious knobs not formed

7) septation percentages based on 12 conidia

knobs at temperatures of 70° F. and above (excluding growth on water agar at 75° F.) indicates the importance of cooler temperatures for inducing formation of trapping structures in pure culture.

Having established corn meal agar as the best substrate tested and 75° F. as a suitable growing temperature, the effect of pH on fungus growth was then investigated. Separate lots of liquid CMA were adjusted to a pH range of 3.3 to 9.2 using 0.1 N citric acid or 0.1 N sodium hydroxide. Necessary amounts of distilled water were added to each container of agar so as to insure that each received the same total amount of liquid additive. After autoclaving, the pH of the agar was again measured. Two petri dishes were poured from each lot of agar and inoculated with *D. drechsleri* when cool. After 3 weeks, the same measurements were made as in the previous test, as well as a final measurement of the agar pH.

A pH range of 5.2 to 6.0 seemed best for optimum lateral growth of the fungus, although growth at pH 8.2 was almost as good (Table II). Maximum conidium length was evident at pH 6.0, but maximum width occurred at pH 3.9. These results indicate that an increase in pH results in the formation of more slender conidia. Maximum knob width seemed to occur at the extremes

TABLE II.

Growth characteristics of D. drechsleri in pure culture on corn meal agar at 75° *F. as affected by* pH.

pH of medium before autoclaving	3.3	3.7	5.0	6.0	7.0	8.1	9.2
pH of medium after autoclaving	3.6	3.9	5.2	6.0	7.9	8.1	8.2
Width of growth zone in mm 1)	— 2)	38	72	64	43	50	63
Relative degree of sporulation	0 3)	+++	+++	++	++	++	++
Average conidium length in μ	—	38.6	34.3	45.4	38.6	36.4	37.9
Average condidium width in μ	—	13.7	12.1	13.3	12.6	12.3	10.1
Average knob length in μ	—	11.0	— 4)	10.5	10.1	10.9	11.0
Average knob width in μ	—	10.7	—	9.5	9.6	9.9	10.2
% conidia-1 septum	—	0 5)	0	7	0	8	8
% conidia-2 septa	—	26	31	7	19	15	38
% conidia-3 septa	—	74	65	67	77	65	46
% conidia-4 septa	—	0	4	19	4	12	8
pH of medium after fungus growth	—	4.0	6.9	7.7	7.2	8.0	8.0

1) average of two replicates

2) very sparse fungus growth; agar failed to solidify

3) Code:

0 = none
+ = light
++ = moderate
+++ = heavy

4) predacious knobs not formed

5) septation percentages based on 12 conidia

of the pH range. It is surprising that predacious knobs were not formed at pH 5.2 whereas they were formed at all other pH ranges. Maximum production of quadriseptate conidia characteristic of *D. ellipsospora* occurred at pH 6.0; however, even at this pH there were 3-1/2 times more triseptate conidia produced, which is a characteristic feature of the new species *D. drechsleri*.

Inspection of Table I indicates that adhesive knob formation in pure culture failed to occur on corn meal agar (CMA) within a temperature of 70° to 80° F. Yet, in Table II, knobs are reported present at all pH values except that where the pH of the medium was 5.2 after autoclaving and prior to inoculation with fungus. In this case, the pH rose to 6.9 after the fungus had grown on the substrate for 3 weeks. Corn meal agar, as it was prepared for these tests, was found to have a pH of 5.8. Thus it seems likely that the pH of the CMA preparations in Table I approximated the pH range of the preparation in Table II, where pH after autoclaving was 5.2 and in which knob formation did not occur. Hence, pH can be implicated as a likely reason for the absence of knobs in CMA at temperatures of 70° F. and above as shown in Table I.

The specific name is proposed in honor of Dr. CHARLES DRECHSLER, whose world-renown research first brought to light the diversity among and potentialities of nematode-trapping fungi.

Dactylella drechsleri, sp. nov.

Mycelio plerumque effuso, hyalino, lente crescente; hyphis sterilibus septatis, 4.0 μ (3.2—5.3 μ) crassis, pediculos rectos uniseptatos 7.9 μ (4.4—14.0 μ) longos et 3.1 μ (2.9—3.5 μ) latos ad pares angulos promittentibus, quoque pediculo bullam tenacem unicellularem 9.0 μ (8.0—10.0 μ) longam et 8.3 μ (7.2—9.6 μ) latam ferente. Bullis ad vermiculos nematoideos inhaerentibus, animalia ita capientibus, integumentum cujusque captivi perforantibus, corpus globosum intrudentibus; corpore globoso mox hyphas filiformes emittente quae vermiculum omnino pervadunt et carnem ejus assumunt. Hyphis fertilibus incoloratis, paulo septatis, 145 μ (93—184 μ) longis, plerumque simplicibus, in apice unum conidium ferentibus, sed interdum subter apicem 1—2 ramulis praeditis qui item conidia singulatim ferunt. Conidiis incoloratis, aliquid fusiformibus, apice late hebetibus vel rotundis, deorsum ad instar coni attenuatis, basi angustis et abrupte truncatis, 39.6 μ (29.5—48.7 μ) longis, 13.1 μ (10.7—15.4 μ) latis, plerumque 3-septatis, aliquando 2-septatis, etiam in statu vetulo rarius 4-septatis.

Mycelium usually profuse, hyaline, slowly spreading; vegetative hyphae septate, 4.0 μ (3.2—5.3 μ) wide, giving rise at right angles to uniseptate, straight stalks 7.9 μ (4.4—14.0 μ) long and 3.1 μ (2.9—3.5 μ) wide bearing spherical to subspherical unicellular knobs 9.0 μ (8.0—10.0 μ) long and 8.3 μ (7.2—9.6 μ) wide. Knobs capturing nematodes by adhesion, with subsequent perforation of integuments of victims. Following penetration of nematode body, a globose assimilative fungus structure formed from which trophic hyphae emanate, rapidly filling body cavity while utilizing nematode protoplasm. Conidiophores hyaline, erect, sparingly septate, 145 μ (93—184 μ) long, each terminating in single conidium but often forming one or two branches, well below apex, also bearing solitary conidia. Conidium hyaline, somewhat fusiform, usually tapering-conical with finely truncate base but broadly obtuse to bluntly rounded at distal end. Conidia measuring 39.6 μ (29.5—48.7 μ) long and 13.1μ (10.7 15.4μ) wide, predominantly 3-septate but also often 2-septate. Conidia bearing 4 septa rare, even in old cultures.

Capturing and consuming nematodes; isolated from grapefruit roots and soil obtained from a grove bordering on U. S. Route 17 south of Wauchula, Florida.

Type cultures of *Dactylella drechsleri* are numbered F1636 in The National Fungus Collections, Plant Industry Station, United States Department of Agriculture, Beltsville, Maryland.

I wish to thank Dr. CHARLES DRECHSLER for preparing the Latin diagnosis for me. He did this without any knowledge that the fungus would be named in his honor.

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Methods for Studying Soil Microflora-Plant Disease Relationships, by L. F. Johnson, University of Tennessee, Knoxville, E. A. Curl, Alabama Polytechnic Institute, J. H. Bond, Clemson College and H. A. Fribourg, University of Tennessee. The Burgess Publishing Company, Minneapolis 15, Minnesota. 1959. 178 pp. $ 3.50.

As an attempt to bring together the many techniques available for the study of the microbiota of the soil this manual was prepared under the auspices of the Technical Committee of Southern Regional Project S-26, titled ,,The Relationship of Soil Microorganisms to the Incidence of Disease in Plants''. Lithoprinted from typescript on pages $8\frac{1}{4} \times 5\frac{1}{2}$ inches, this spiral bound volume is clearly reproduced, well illustrated and relatively free of serious typographical error. It is well documented with a list of 237 references following the 15 chapters each of which treats a special aspect of the study of soil microorganisms.

Several techniques are described for taking soil samples, but it is emphasized that for each use, or even each soil, sampling techniques may be altered. Detailed techniques are given for the isolation of microorganisms from soil. A general technique is given in detail for soil dilution and plate counts, and several modifications are described, together with a listing of the agars used for fungi, Actinomycetes and for bacteria and/or Actinomycetes. Descriptions of special techniques for isolating fungi from soil include direct inoculation, the soil plate, the screened immersion plate, the immersion tube and its modifications, the isolation of fungal hyphae from soil and a special technique for the isolation of Ascomycetes from soil. Techniques are also described for the isolation of algae, amoebae and bacteriophages from soils.

Several procedures are given for qualitative and quantitative studies of soil microorganisms without isolation in pure culture. These include the buried slide, the direct microscopic examination of soil, the counting of soil algae by direct fluorescence and a flotation technique.

For the study of microorganisms in the plant rhizosphere, soil dilution and plate count techniques are given and are supplemented by serial root washing, the soil plate, the soil box, the contact slide, and the miniature root observation box. For the establishment of an artificial rhizosphere the collodion membrane technique and the sterile sand technique are described.

Soil microbial populations can be characterized taxonomically or by nutritional, ecological or antagonistic groupings. Each technique is described in detail. Antagonistic soil microorganisms can be isolated by random isolates in pure culture, the bacterial agar plate, layers of agar separating one or more potential populations, one of several spray techniques, or by the colonization of sclerotia.

Tests of microorganisms for antagonism in agar culture are listed and illustrated. Types of reactions and criteria for antagonism are described. Following suggestions for selection of a test method, table 1 presents a list of about 100 test organisms, test antagonists and the method used in making the tests.

The techniques offered for the assay of antibiotics and of sterile culture filtrates are the dilution method, wherein three different types of dilution tests are described in tables, the cylinderplate or cup method, and the paper- disc plate method.

The growth and survival of microorganisms in the soil are studied in sterilized soils or through the use of glass slides, glass tubes, cellophane, agar discs, or fiberglass. Each technique is described carefully, with its currently used modifications. The morphological form or condition of microorganisms in the soil is considered important to the understanding and interpretation of the qualitative and quantitative expression of soil populations.

Soil solutions are prepared by water extraction, displacement, a pressure-membrane apparatus or a soil perfusion apparatus. Techniques for determining the production of antibiotics in soils include extraction procedures, percolation, the soil-sandwich technique, and the buried slide. Methods to determine the persistence or inactivation of antibiotics added to the soil are listed.

Interactions between plant roots and soil microorganisms are studied by the extraction of chemicals from plant parts. Rhizosphere effects on microorganisms are studied by use of a cellophane bag technique, the collodion membrane sack technique and the sterile sand technique. The chemical nature of root exudates is studied by the use of a filter paper technique or an artificial rhizosphere. Six techniques are given for the extraction of chemicals produced by soil microorganisms.

The utilization of antagonistic microorganisms for the control of soil-borne plant diseases is described. Cultures of such organisms can be added to the soil, antibiotics or culture filtrates can be used, seeds can be treated with cultures of antagonistic organisms, organic amendments can be added to the soil, and crops can be rotated. Possible chemical interactions are described in two cases.

An extensive chapter on data analysis, experimental design and statistical procedures is included. It is the only chapter with an independent bibliography. Finally a chapter is given listing formulae of selected media. These are divided into all purpose media, media for the isolation of soil microorganisms, fermentation media for antibiotic production, culture solutions for higher plants, and media for the inoculation of soil with parasitic or saprophytic microorganisms.

That the authors relied almost exclusively on the literature of plant pathology and of soil microbiology is quite apparent. For instance, the flotation methods used in searches for fungi pathogenic for man from soil samples are not described, although they could be useful to some users of this book. Despite its restriction to the study of organisms pathogenic for plants, this volume can have wide application in the field of microbiology in general and should be of use in any laboratory dealing with microbiological problems.

Wm. Bridge Cooke

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Kamat, M. N.: Hand Book of Mycology. Part I: Phycomycetes & Ascomycetes. 184 pp., 51 plates. Cloth, crown size. Prakash Publishing House, 360 Budhwar Peth, Poona 2, India. Price 20s.

This handbook is basically a manual for postgraduate students in mycology and also for the teachers showing a well conceived methodology and system in presenting the subject. The author is a renowned teacher of over 35 years' experience. This handbook is a combination of a text and a laboratory guide. It is profusely illustrated with Indian material. It discusses all the important mycological topics. The Indian literature and monographs are emphasized.

The handbook presents the material in 45 ,,exercises". Well balanced, the first part of the volume deals with Phycomycetes, the second one with Ascomycetes. In both sections, first the general characteristics of the Families are given, than the laboratory instructions for the student follow with laboratory exercises in the important genera. To every ,,Exercise" well selected references are added to enable the student to dig deeper in the material of his special interest. Several useful appendices on mycological technique, on keys to important genera, monographic and general references close the handbook. The text is well written, clear and concise with most instructive illustrative material. In the references the Anglosaxon and particularly the American literature is exhaustively considered. The production of the book is highly satisfactory. Due to the highly original, didactic presentation of the material this hand book is warmly recommended both for the students and teachers in mycology.

Tibor Benedek

Frederick K. Sparrow, Jr., *Aquatic Phycomycetes*, Second Revised Edition. The University of Michigan Press, Ann Arbor. pp. xxv, 1187, 91 text figures. 1960 $ 22.50

The first edition of Sparrow's book, published in 1940, has been the principal guide for the students of the chytrids and other aquatic fungi. The second edition brings together in one volume descriptions of all species mentioned in the first edition, brings up to date by reference, at least, those species of the Saprolegniaceae not included in the Coker and Matthews monograph (North American Flora 2 (1): 1-76, 1937), and adds certain groups to round off the complete picture of those fungi known to be restricted to water or aquatic habitats.

The book was printed in The Netherlands by E. J. Brill, Leiden, on fairly heavy paper, making it rather bulky. It is relatively free of typographical errors and the plates are clear. A 31 page "list of substrata" is set up as a list in this edition in contrast to the paragraph style used in the first edition. In this way it is much more usable. It appears that members of all groups of algae, many Phycomycetes and Ascomycetes among the fungi, three species of bryophytes, seven pteridophytes, the pollen of many species of gymnosperms and angiosperms, a number of mature species of angiosperms, the woody parts of both gymnosperms and angiosperms. fruits, soft tissues and seeds of various higher plants, numerous kinds of invertebrate animals and numerous types of organic substances are host to the many kinds of organisms treated in this volume. A 60-page bibliography containing more than 1300 titles completes the book.

The introductory chapter indicates briefly the position of the Phycomycetes among the fungi and lists the orders into which the class is divided. These orders are arranged in three series: those which are zoosporic and aquatic exclusively, those which are zoosporic or "conidial" and which occur terrestrially or as amphibians, and those which are exclusively conidial which are terrestrial except for one genus. Treated in this book are all the aquatic members of the three groups. Phylogenetic relationships are summarized. The zoospore is described in detail and seven principal types are illustrated. The flagella are described, the whiplash and tinsel types are illustrated, and it is shown how each of the different groups of aquatic Phycomycetes has its own peculiar type of flagellation. A brief section on occurrence is followed by a more extensive discussion of the hydrobiological aspects of these fungi. Geographic distribution is discussed briefly. It is noted that the class is ubiquitous although certain groups are more common in tropical soils than others, and numbers of genera in a habitat fall off as one approaches the North Pole. A comprehensive discussion of collecting techniques is followed by an extensive description of techniques used by different specialists within the group for isolation and culture of these organisms which are usually more fastidious than other types of fungi. A key to the ten orders of Phycomycetes completes the introduction. Key characters are broad enough to include members of these orders not treated in the text of this volume.

For each of the ten orders treated, detailed information is given concerning historical background, development and morphology, reproduction and cytology. The discussions of morphology and cytology are well illustrated. Since the Chytridiales is the largest order treated, it is natural that this order is described most fully. Establishment and development of the thallus is divided into four types: the *Olpidium* type, the *Entophylyctis* type, the *Chytridium* type and the *Rhizidium* type. The general structure of the thallus is described in detail. Reproduction is divided into a discussion of non-sexual and sexual reproduction. Cleavage of the zoospores, their discharge, their structure and behavior, and their germination are considered especially in reference to deviation from the usual type of activity. Sexual reproduction is considered in detail, diagrammed, and illustrated. The parasitism of chytrids is discussed, the chytrid epidemic is considered and saprophytism and facultative parasitism are discussed. It is thought that it cannot yet be definitely stated that host specificity is of importance since few studies have been made of this characteristic. The effect of the chytrid on the host cell and the resistance of algae to chytrid infection are discussed in the light of present knowledge based on observation and speculation concerning observed structures. It is suggested that most cases of hyperparasitism are based on erroneous reports or identifications of organisms found living together or observed in samples.

Discussion of morphology, cytology and reproduction in the Blastocladiales is based largely on work on *Allomyces* published by EMERSON and WILSON. Because this is among the best known Phycomycete genera in these regards this is a lengthy and valuable preface to the systematics of the order.

Since this book is largely a systematic account of an important segment of fungal species, an analysis of the numbers of species treated in each of the 10 orders is considered pertinent. There are 9 flagellate orders and 1 conidial order. The flagellate orders are divided into "uniflagellate" and "biflagellate" orders. Of the uniflagellate orders, the Chytridiales is the largest order with 9 families arranged in 2 series. Sixty-three genera are considered in which are placed 385 recognized species. In addition 2 forms and 2 varieties are recognized. Nine imperfectly known genera, and 131 imperfectly known species distributed among recognized genera as well as among excluded, rejected, or imperfectly known genera, are described or otherwise discussed to the extent that such comments are useful. Twenty-three species are rejected or exluded. The text of this chapter includes 1 new genus, 1 new name, 5 new species, and 21 new combinations.

Three families are recognized in the Blastocladiales. These include 7 genera, 48 species and 3 varieties. There are 4 imperfectly known or rejected species and 1 hybrid species (in *Allomyces*). In the Monoblepharidales 2 families are recognized. These include 3 genera, 16 species, 3 varieties, 1 excluded species and 1 rejected genus. Three families are placed in the Hypochytriales and to these are assigned 6 genera, 14 species, 1 imperfectly known genus, 2 imperfectly known species, 1 excluded genus and 1 species of doubtful affinity. Two new genera and 2 new combinations are included.

There are 5 biflagellate orders. These include the Plasmodiophorales with 1 family to which are assigned 7 genera, 18 species, 2 imperfectly known species and 1 imperfectly known genus. In addition certain species which are known to attack aquatic plants are cited but not described.

The species descriptions in the Saprolegniales are confined to those groups not listed by COKER and MATTHEWS (l.c.). Three families are considered with 24 genera, 54 species, 3 imperfectly known species and 2 imperfectly known genera. For those species in the Saprolegniaceae not considered by COKER and MATTHEWS citations are given, and a recent publication by T. JOHNSON (1956) is considered sufficient reference to species of *Achlya*.

Two families are assigned to the Leptomitales and these include 7 genera, 20 species, 2 varieties and 1 excluded species. Two names are given a new status in this order. In the 3 families of the Lagenidiales are placed 8 genera, 64 species, 1 variety, 1 form, 18 imperfectly known species, 1 excluded species, 1 doubtful genus with 3 species and 2 imperfectly known genera each with 1 species. One new name and 1 new combination are included.

In the Peronosporales, an order which includes the most important plant parasites belonging to the Phycomycetes, only one family is included, the family which has species whose zoospores are flagellated. Five genera with 8 species, 1 excluded species and 4 imperfectly known genera are considered. A key, extracted from MIDDLETON's key (Me. Torr. Bot. Cl. 20: 1—171, 1943) with recent additions, is given to 37 species of *Pythium* parasitic on water plants or known to occur in water. Five species of *Phytophthora* parasitic on aquatic plants or occurring on plant parts in water are described.

The order including only conidial species is the Entomophthorales. One family is considered and in it only the genus *Ancylistes* which has three species parasitic on desmids. In addition 1 imperfectly known species and 3 excluded species of this genus are mentioned.

Two comments are in order concerning taxonomic techniques. In several places a species is cited as "pro parte". According to the International Code of Botanical Nomenclature, a species is based on a type. If the type contains more than one element, only the element chosen by the monographer can be considered the type of the described species. Thus the final description can apply only to that portion of the type selected by the author as being representative of the original concept. Thus if the method of selecting the type specimen is described adequately in the discussion, the "pro parte" is superfluous.

The species, not the typical variety of the species, when more than one variety is proposed, is credited to the original and combining authors. Thus it seems to this reviewer that *Phlyctidium brevipes* var. *brevipes* (ATKINSON) MINDEN (p. 221) is more correctly cited as *Phlyctidium brevipes* (AKTINSON) MINDEN var. *brevipes*. No authority name should follow the second *brevipes*, but to prevent the appearance of a trinomial the "var." should be included. The complete citation, then, for the second variety would be *Phlyctidium brevipes* (ATKINSON) MINDEN var. *marinum* KOBAYASI and OOKUBO. In citing varietal taxa throughout the book the earlier form was used consistently except in several cases where the recognition of the typical variety was omitted.

Apparently, cost and space considerations made it prohibitive to include all species of aquatic fungi, especially when monographs describing the other species are readily available to most laboratories.

This text will be a most useful tool of the aquatic biologist dealing with the fungi. It will be a very useful book in the mycology laboratory where the student is learning the different types of fungi and how to identify them. It will aid the student of aquatic fungi in the identification of almost any fungal material he finds in aquatic habitats whether these are saprobic or parasitic.

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JAEGER, E. C.: The Biologist's Handbook of Pronunciations. Illustrated by M. VAN DAME and the Author. 317 pp. Charles C. Thomås, Publisher, Springfield, Ill. USA.

This handy pronouncing guide includes over 9,000 commonly used and often mispronounced technical terms. With each specific name is given its original Greek or Latin meaning or Englisch equivalent. For ready reference a table shows the needed diacritical marks. Sixty-two drawings, beautifully executed by the Artist MORRIS VAN DAME and the author call particular attention to words often mispronounced. With the rapidly declining and mostly non-existing knowledge of Greek and Latin this guide fills a definite gap. Its wide usage and frequent consultation is urgently recommended.

TIBOR BENEDEK

Illustrated Genera of Imperfect Fungi, 2nd Edition, H. L. BARNETT, West Virginia University, Morgantown, West Virginia: Burgess Publishing Company, Minneapolis 15, Minnesota 1960. 225 pp. $ 4.50.

This publication was conceived by the author a number of years ago after he prepared a few pages of illustrations for mycology students at West Virginia University. These pages demonstrated the value of illustrations and he proceeded to expand the material into that of a manual publishing the first edition in 1955 containing 302 genera out of an estimated 1000 genera. He made an effort to place emphasis on common or important genera but has included a number of unusual forms.

Most of the drawings are original with the author, were made free hand by him and the remainder were traced from original illustrations, the author crediting the source in case the latter was true. He stippled hyphae, conidiophores and conidia to indicate the presence of dark pigment while hyaline structures are not treated this way. The appearance of the fungus is shown under different magnifications, the low magnification drawings made by use of the stereoscopic microscope, high power or oil immersion views were used for high magnification drawings.

The 2nd revision includes a total of 462 genera, an increase of about fifty per cent over the 1st edition. He has especially included a number of genera from foreign countries because of the worldwide interest in imperfect fungi. Twenty-four genera of Conidial Phycomycetes which are similar to the Moniliales, are followed by the Moniliales, Sphaeropsidales, Melanconiales and Mycelia Sterilia. The authorities for the generic names are taken from AINSWORTH & BISBY and for the most part, synonyms have been omitted. The author has stressed the fact that arrangement of genera are based primarily on descriptions of spore characters such as color, shape, number of cells and in some cases characters of the conidiophores. As usual one finds a Key to the Included Genera followed by descriptions and illustrations, then references and finally the index.

This manual is intended primarily as a manual for the student, with brief descriptions and illustrations to facilitate recognition and identification of some of the imperfect fungi. Students of general mycology as well as those in medical mycology should find the manual very useful. Descriptions of some fungi pathogenic for man and animals have been included. The reviewer might suggest that since the author has included drawings of bud cells produced in tissue by the dimorphic, pathogenic fungus *Blastomyces dermatitidis*, that he also include budding cells produced by *Histoplasma capsulatum* and *Sporotrichum schenckii* for the sake of uniformity. Also would it not be a good idea to include a glossary? Most students find it a very useful adjunct to their understanding of a subject filled with confusing terminology.

Outside of these few criticisms, the reviewer considers it a very useful manual for all students of mycology as intended by the author.

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M. WELSCH, Die bakteriologischen Untersuchungen und die Resistenzprüfungen gegen Antibiotika. Translated into German by W. ROTH. pp. 98. Georg Thieme, Stuttgart, 1960.

This manual is one of a series entitled "Diagnostik." Its author is a member of the faculty of the Medical School of the University of Liège, Belgium. It has three purposes: 1) to instruct the physician in the types of services that he may expect from a clinical bacteriological laboratory, 2) in the proper collection of specimens for bacteriologic examination, and 3) in the general technics of bacteriologic examination. Those technics which are most practical for clinical practice are stressed. Technics for the evaluation of the antibiotic sensitivities of bacteria are given in some detail. The only important technic of clinical bacteriology which is not mentioned is the quantitative urine culture. Methods for mycologic and parasitologic examination are not given, although they are alluded to. Although some technics are referred to eponymically, no references are cited.

This booklet achieves its limited purpose quite well and should find popularity among German speaking interns and resident physicians.

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SCHABINSKI, G.: Grundriss der Medizinischen Mykologie. Mit 126 Abbildungen im Text und 5 farbigen Tafeln. 183 pp. VEB G. Fischer Verlag, Jena, 1960. Price: DM 34,20

This excellently written introduction into Medical Mycology should serve the purposes of the laboratory personnel (student, physician, assistent). The understanding of mycological technique and diagnosis is made easy by the well selected illustrative material: clinical photographs, photographs of giant cultures in black and white, and in color as well as the photomicrographic details of fungi discussed. Technically this introduction is divided in four parts: 1) General mycology; 2) European fungus infections; 3) Tropical and subtropical mycoses; 4) Contaminants and Formulae (culture mediums, staining technic, etc.). Every chapter has a well selected bibliography not only of the German, but also the French and American publications. Those in command of the German language may consult this introduction with definite profit. The production of the book is excellent.

TIBOR BENEDEK

Bernard S. Meyer, Donald B. Anderson & Richard H. Böhning: *Introduction to Plant Physiology.* D. Van Nostrand Co., Inc., Princeton, N. J. 1960. 541 pp. $7.50.

This book is a condensed version of Meyer & Anderson's perennially successful textbook, *Plant Physiology*, first published in 1939 and now in its second edition (1952, 784 pp.). It may be expected to be more useful than the longer text in typical one-semester courses in plant physiology.

An effort has been made to bring the book up to date in areas, such as photosynthesis and metabolism, in which considerable progress has been made in recent years. This effort has not, in my opinion, been entirely successful; the organization and spirit of the book have not kept pace with the times, and even though much new material has been inserted, many passages of the present book are similar if not identical to those in the original textbook. Outdated organization gives rise to problems like that on p. 200, where reference is made to phosphoglyceric acid and other compounds as involved in photosynthetic carbon fixation, without the student having any background yet about metabolism, into which the compounds or their conversions could be fitted to make them interesting and plausible. When the related processes of glycolysis are covered later (p. 283) photosynthesis is not brought into perspective.

The treatment of metabolic processes tends, in my view, to be lifeless and disjointed, and to convey little feeling for why we think the various metabolic sequences outlined occur or are important. The discussion of carbon fixation in photosynthesis, about which so much elegant work has been done, seems particularly disappointing. I deplore the presentation of metabolic pathways as sequences of long, unfamiliar chemical names connected by arrows, without any structural formulae to help the student visualize the compounds involved or their relationships.

While the aspects of physiology tied most closely to modern developments in biochemistry are the ones which fare most poorly, inadequacies are not confined to these areas. For example, the discussion of mineral ion absorption makes no mention of active transport or carriers. The chapters on water relations, though occupying almost a third of the book, show little indication of any advance over the static, dry and non-analytical approach to this subject of the thirties.

This volume will undoubtedly be useful to teachers who have used Meyer & Anderson's *Plant Physiology* and are not anxious to teach the subject from a more modern frame of reference. Those who do have this desire will most likely not be satisfied with the book.

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CHARLES L. FERGUS, Illustrated genera of Wood Decay Fungi, 132 pp., 81 figs. Burgess Publishing Co. Minneapolis, Minn. 1960. Price $4.00.

This publication is the third in the Burgess Life Science Series; all three so far published deal with particular groups of the fungi. Certainly it will be as favorably received as the two preceding it, *Illustrated Genera of Imperfect Fungi* by H. L. BARNETT (ed. 2, 1960) and *Illustrated Genera of Rust Fungi* by GEORGE B. CUMMINGS (1960). Dr. FERGUS states that he hopes his manual "will fill a definite need, that of the general forester to identify decay fungi", and that it will also be useful in Forest Pathology laboratory courses. In this reviewer's opinion, Dr. FERGUS has been too conservative in indicating the possible uses of this manual. Not only the general forester and the college student enrolled in Forest Pathology, but also Botanists and Naturalists, as well as Mycologists, will want to add this publication to their libraries.

In a short introduction the economic importance of wood decay fungi is discussed, the use of the dichotomous keys is explained, and brief directions for the collection for wood decay fungi are outlined. The keys fall into four categories. The first key is to the families of basidiomycetous fungi causing wood decay. It utilizes both macroscopic and microscopic characters. This is followed by a generic key based entirely on macroscopic characters. Separate keys to the genera of each family (except Polyporaceae) are also provided. Finally, for genera containing more than a few important species, keys are included. For the Polyporaceae a single key to species has been prepared. A concise diagnostic description for each of the 85 genera is given; the black and white illustrations provided for 81 genera are excellent. As acknowledged by the author, the keys are based on those developed by L. O. OVERHOLTS and used both by him and his students (including this reviewer) for many years at Pennsylvania State University. Over the years the keys have been constantly revised to increase their usefulness. Several species which are perplexing to the neophyte are keyed out at two or more different points so that a correct determination can be reached even though a "wrong" choice is made at one branch in the keys. A list of well-chosen general references, a glossary and an index complete the manual.

A few ascomycetous genera, such as *Xylaria*,*Daldinia*, and *Ustulina* cause wood decay. The omission of these genera actually results in the manual being a treatment of only the basidiomycetous genera causing wood decay. Species belonging to such genera as *Auricularia*, *Exidia* and *Tremella*, although they do not initiate wood decay, are consistently found on decaying wood and play an important role in decomposition. Consequently, there is every justification for Dr. FERGUS' including them in a manual on wood decay fungi. On the other hand, species of *Septobasidium* (occurring on the bark of trees, but parasitic on scale insects), of *Exobasidium* (on leaves and twigs of members of the Ericaceae) and of *Phlogiotis* (found on the ground under conifers) are not normally associated with wood decay and are apparently included only because of their sylvan habitats and the fact that they are Basidiomycetes.

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M. Faure, R. Pautrizel and L. Leminor. Die serologischen Reaktionen bei den parasitären und infektiösen Erkrankungen. Translated into German by W. Roth. pp. 80. Georg Thieme, Stuttgart, 1960.

This manual is another in the series entitled ,,Diagnostik." Its principal author is in charge of the teaching of serology at the Pasteur Institute. The first portion is devoted to an explanation of what a serologic reaction is, what the main types of serologic reactions are, and what diagnostic significance such reactions have clinically. For a presentation of such brevity the exposition is very satisfactory.

The other three fourths of the manual consider the serologic reactions which are appropriate for the investigation of human infectious diseases of bacterial, viral and parasitic etiology. Serologic examinations which pertain to mycoses are only mentioned. The considerations required for the interpretation of the various tests are emphasized, while details of the technics are largely lacking. There is no bibliography.

This manual is a source of rapid and useful reference for the German speaking physician-in-training as well as in practice.

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MICROBIOLOGY: Historical Contributions from 1776 to 1908. Edited by R. N. Doetsch, Rutgers University Press, New Brunswick, N.J. 223 pp., illustrated, 1960. Price $ 5.- -

History of the natural sciences in any of its branches should be known and better known by the active workers than usually it is the case. The history of science has been and is concerned with ideas as they are logically related to and arising from the conditions of time and circumstances. This well edited book brings great original contributions to the development of microbiology over a period of 132 years. Almost every one of these papers represented in its own time and place a break through the frontiers of knowledge into new and previously unchartered areas. The men and their work represented in this volume are mile stones along the hard road of development of microbiology. This volume contains contributions of SPALLANZANI, SCHWANN, PASTEUR, COHN, TYNDALL, KOCH, LISTER, SCHLOESING, BURRILL, EHRLICH, WINOGRADSKY, WARINGTON, BEIJERINCK, SMITH and ORLA-JENSEN. To every contribution the author's portrait is added. Moreover, there is at the end of every contribution a well selected list of readings for those who want to have more detailed information about the single problems. The old adage: ,,Historia est magister vitae" holds true also for the natural sciences, revealing the birth of ideas, the hard way, the great struggle they go through to ultimate victory. This volume is warmly recommended to all microbiologists.

TIBOR BENEDEK

HERB. I. M. I. HANDBOOK, Methods in use at the Commonwealth Mycological Institute, edited by J. C. F. HOPKINS. Commonwealth Mycological Institute Ferry Lane, Kew, Surrey, 1960. 103 pp. Price 12s.6d.

This concise volume brings the practices of the ,,Herbarium, Imperial Mycological Institute" (Herb. I.M.I). Thirteen members of the Institute Staff participated in writing this ,,Handbook". After an introduction by the present director of the Institute, J. C. F. HOPKINS, WILTSHIRE presents the history and development of I.M.I. The Herbarium is described by ELLIS, and DEIGHTON discusses the method, how to describe a fungus. The standard laboratory procedures used at I.M.I. are presented by DADE. The pathogenic Phycomycetes are treated by WATERHOUSE, Bacteria and the Herbarium by HAYWARD. The methods of collecting fungi are presented by BOOTH, DEIGHTON and AINSWORTH. DEIGHTON discusses the ,,Index of Fungi" and WATERHOUSE the ,,Bibliography of Systematic Mycology". MARTIN reports on the Review of Applied Mycology and AINSWORTH on the Review of Medical and Veterinary Mycology. The volume contains, furthermore, a list of the present Staff of I.M.I. and a useful list of its publications. Six photographs and eight line drawings illustrate the volume. This ,,Handbook" contains not only the history of one of the world leading Mycological Centers, but it brings a treasure of the most useful information. The physical make-up of the volume is excellent. It should be at hand on the shelf of every mycological working place.

TIBOR BENEDEK

Sadler Moss, E. & McQuown, A.L.: Atlas of Medical Mycology. Second edition, 335 pp. Williams & Wilkins Co. Baltimore, 1960. Price $11.-

Seven years after the first edition and two reprints the second edition of this work made its appearance. The title is somewhat misleading, for this work is far more than a usual atlas used to offer. It is a most comprehensive presentation of medical mycology. It is introduced by a useful "Glossary" with proper spelling (particularly for those whose knowledge of Latin is somewhat wanting) and short explanation of terminology peculiar to mycology. In thirty chapters the whole breadth and depth of medical mycology is covered. The material is first presented by "Classification of Fungi" in descriptive terms along with exhaustive synoptic illustrative material. Every chapter states first the etiologic agent, than the definition of the disease entity, the clinical forms, laboratory diagnosis, mycology, histopathology (both authors are pathologists by their backgrounds) and a short description of treatment and prognosis.

Despite the fact that there are at least several equivalent treatises on medical mycology, this work is simply not "one more" among its peers. It does not contain anything more than other similar texts do, but the concise, clear didactic presentation of the whole material gives a uniqueness to this work. Several synoptic, detailed tables on dermatomycoses, classification, key to fungous infections will be most useful for the students. Every pathogen is described and illustrated as a giant culture along with its microscopical morphology and with its appearance in histological section. A special chapter is consacrated to the mycological technic. Others are dealing with immunology, with contaminants (outstanding), with preparation of the most useful culture mediums and with a chapter on special stains for fungi. Thirteen, mostly colored, plates, a courtesy of the Upjohn Company, enhance the value of the "Atlas".

Where there is too much light, there is also plenty of shadow. This holds true also for this publication. Since both authors are pathologists first, one could have expected that they will apply the stern scrutiny of their basic discipline also to the etiologic and pathogenetic evaluation of certain "pathogenic" organisms in definite entities. In this respect they completely failed. They could not rise above the deadly conformity of any of the current mycological texts. Onychomycosis, otomycosis, tinea pedis are caused by *Candida albicans* and a number of hyphomycetes. One clearly feels, that in these most important dermatological entities the authors are completely lacking the necessary first hand information. There is not a word about the primary lesion of the involvement of the nail organs; not a word about the primary lesion, the deep seated sagograin-like blister, in "tinea pedis", entities whose etiology and pathogenesis have nothing to do with *Candida albicans* or any of the hyphomycetes. The authors claim their evidence by holding up "pure cultures" of the different microorganisms which were found in the detritus masses of the skin lesions. Even repeated "pure" cultures are not the final evidence for their etiologic and pathogenetic role. And the authors have the finest, most adequate method at their finger tip to cut through the detritus masses of this Augian stable. They did not use it and thus, became the victims of misleading dermatologic "advice". It is useless to go more in detail in this matter since the authors did not know or have completely ignored all the progress achieved in etiology and pathogenesis concerning onychomycosis, otomycosis, "tinea pedis" the last thirty years. They are not worse or more neglectful than the authors of any other similar texts reviewed in these pages through the years. But since they are pathologists, they have no excuses for these errors and omissions.

Concerning the illustrations what the authors present under Candidiasis" on pages 71—74 are classical forms of seborrheic dermatitis, an endoparasitic-bacterial affliction. On page 72 the involvement presented as "Candidiasis" is again not a "Candida" infection, but pompholyx of the nail organ, of endoparasitic-bacterial origin. Similarly erroneous are the captions of the illustrations on page 212 representing "dermatophytosis". The expert sees it at first glance that the illustrations represent nummular eczema and lichen Vidal, both skin manifestions of hypovitaminosis A.

There are few errors in spelling. On page 169 in the caption it should read "flask" instead of "flash". On page 153 it should read "Mucor corimbifer" and not "corimbifera". On page 187 the authors consistently use "detritis" where the word is "detritus". On page 180 contrary to the spelling rules of the International Code of Botanical Nomenclature they write "Microsporum stillianus". Since the nomen proprium is "Stillians", the genitive of the word is "stilliansis".

Of course, these small errors can be easily eliminated in the next edition, which the reviewer hopes will be forthcoming in due time. The physical appearance (print, paper, illustrative material) of the work is impeccable. There is a well selected bibliography which, however, contains only American publications.

Despite the fact that the authors were unable or unwilling to break through the iron wall of deadly conformity blanketing medical mycology what they as pathologists would have been expected to do, this book, nonetheless, is warmly recommended for the beginner and also for the advanced student in medical mycology on account of the unique didactic presentation and of the rich illustrative material.

Tibor Benedek

DEIGHTON, F. C. African Fungi. I. Mycological Papers, No. 78. Commonwealth Mycological Institute, Kew, Surrey. pp. 43. 2 plates, 22 text figures. 1960. Price 12s. 6d.

A number of different fungi are discussed, mostly Hyphomycetes but including a few Ascomycetes and one genus assigned to the Mycelia Sterilia, having in common their African origin, mainly Sierra Leone. Four new genera, 11 new species and 2 new combinations are proposed.

The new genera, *Megalodochium* (p. 17), *Helicominopsis* (p. 20), and *Hansfordiellopsis* (p. 33) and *Ampullifera* (p. 36), all assigned to the Hyphomycetes, are fully described and well illustrated. Other genera discussed are *Tretopileus*, *Memnoniella*, *Linotexis*, *Hansfordia*, *Sclerographium*, *Trichothyrium*, *Hansfordiella*, and *Xylohypha*.

The disseminules of *Monotospora sphaerophora* BERK. & CURT. are found to be gemmae, not conidia and this provides an earlier epithet for *Tretopileus opuntiae* B. O. DODGE. BERKELEY and CURTIS described it very briefly and assigned it to the Dematiaceae; DODGE described it in great detail, with excellent illustrations, but did not place it in any taxonomic group. The fungus, under its new name, *Tretopileus sphaerophora* (BERK. & CURT.) HUGHES & DEIGHTON, is assigned to the Mycelia Sterilia and, it is suggested, may prove to have a wide distribution in the tropics. The treatment of *Xylohypha* merely legitimatizes MASON's raising of a subgroup of *Hyphelia* FRIES to generic rank, and does the same for its three recognized species.

It is impossible in the brief space available to cover all of the topics treated. It is to be hoped that from time to time these notes will be summarized in systematic form. Meanwhile, anyone concerned with the increasingly intricate classification of the fungi cannot afford to neglect the valuable and suggestive contributions appearing in this series.

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Gallery of Contemporary Noted Mycologists

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Gallery of Contemporary Noted Mycologists

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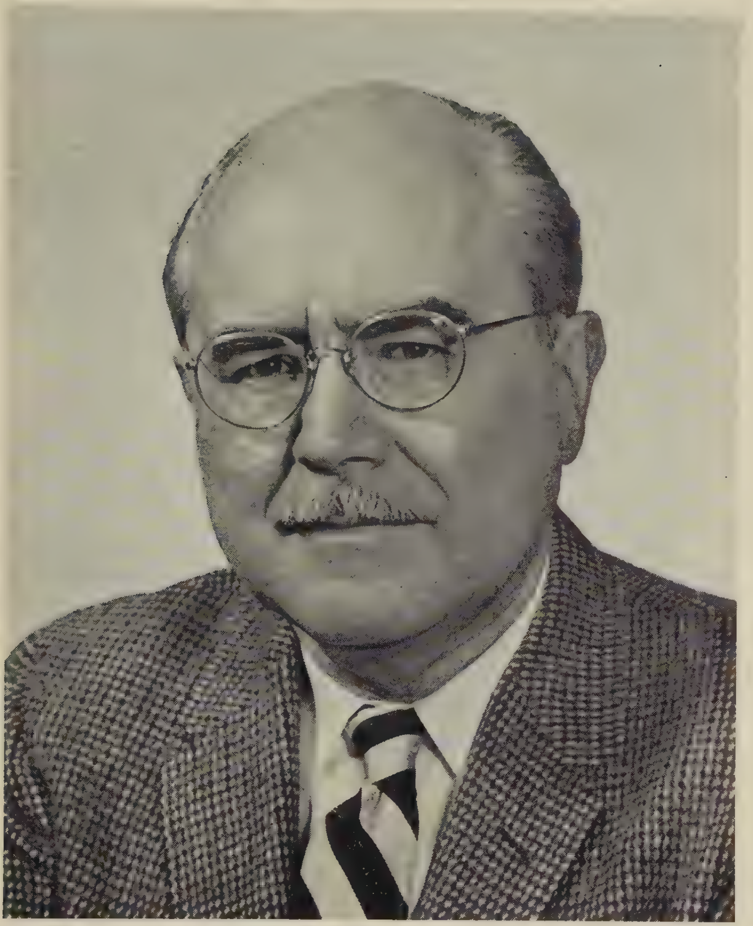

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AN ESSAY ON THE CLASSIFICATION OF MONILIACEOUS MEDICAL FUNGI

by

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(23.X.1960)

Up to the beginning of this century, most of our knowledge of the fungi found on man (and animals) has been limited to the description of parasitic fungi with little regard to their pathogenicity or, at least, to the relationship between fungi and diseases caused.

The first half of the XXth century has been characterized by a thorough revision of our previous findings, with emphasis on the true pathogenic fungi causing clinically well defined diseases. During the second quarter of this century, extensive studies on the comparative morphology, physiology, biochemistry, pathogenicity and immunology have considerably restricted the field, so that only a few dozens species are now considered in standard treatises of medical mycology. Many doubtful entities observed but not cultivated, or incompletely described or duplicating previously known species, have been therefore disregarded.

In spite of this revision, comparatively little effort has been made to draw an organic classification of the pathogenic fungi with regard to the general taxonomy of fungi.

The apparent reason is the conflicting views of the classical, Saccardian system of Moniliaceous (Hyphomycetous) fungi, against the Vuilleminian genial but incomplete classification, discriminating different kinds of the vegetative spores formerly considered as "conidia".

This chasm has been widened by the progress of the mycology along the Vuilleminian line (see, e.g., Mason 1933, 1937, 1941; Hughes 1953; Tubaki 1958), while the regretted French mycologist himself, up to the last treatise (Vuillemin, 1931), several times changed the concept of the categories of vegetative spores.

The confusion is greater for taxa of rank higher than the genus due to the lack of correspondence between the families and orders codified by Saccardo and those established by Vuillemin. This is the reason for the perplexity of several workers (e.g., Nannizzi, 1934; DODGE, 1935; COUDERT, 1955) in the subdivision in tribes and families.

In our opinion the time is ripe for an attempt towards a more complete and logical system of medical fungi in the general systematic mycology. A few preliminary remarks should be made.

In accordance with the traditional rules of taxonomic mycology, the outstanding criterium of classification is - and must remain - the morphological one. But, in disagreement with most of the saprophytic species, many "animalized" pathogenic fungi possess two morphologies, in many cases widely different. One is the morphology in culture on laboratory routine media and room temperature, or, in any way, under conditions approaching the saprophytic life in nature. The other is the morphology of the pathogenic fungi in living human or animal tissues, or cultivated on media approaching the living tissue fluids and at about the same temperature of the human body.

In a previous paper, CIFERRI & REDAELLI (1953) discussed the problem of the morphological life cycle of pathogenic fungi in the saprophytic and parasitic stages, establishing the following categories:

1) Fungi in which the greatest evolutive morphology[1]) of the life-cycle is reached in tissues of man or receptive animals, while in laboratory, on the usual culture media and at room temperature, the morphology is simplified, sometimes incomplete. Only when the conditions (culture media, temperature, etc.) are approaching those of living tissues, certain morphological features or the entire life-cycle may be reproduced "in vitro". This category has been named biotelocarpous fungi, the best example being *Coccidioides immitis*.

2) Fungi in which the greatest evolutive morphology of the life-cycle is reached in routine culture media at room temperature. In the animal tissues, only regressive morphology of little taxonomic significance is found. Most pathogenic fungi are referred to this category, called saprotelocarpous fungi, e.g. *Rhinotrichum* (*Sporotrichum*), *Candida*, a number of Trichophytonaceae (Dermatophyta), and so on.

3) An intermediate group in which the morphology is identic or almost so "in vitro" and "in vivo". Several fungi pertain to this category, denominated hemitelocarpous fungi, as, e.g., Mucorales, Aspergilli and so on.

An example of the perplexing taxonomical situation caused by the different degree of adaptative morphology is that of the genus *Histoplasma*. There is no doubt that the highest morphological expression is the presence, in the saprophytic stage, of the verrucous or thorny aleuriae, resembling those of the genera *Sepedonium*, *Mycogone* and so on. But the expression of the parasitic life is the bulk of blastospores, enclosed in the cells of the reticulo-endothelial system. Taking in consideration the saprophytic morphology, *Histoplasma* pertains to a family of aleurosporeous (Vuilleminian taxonomy) or moniliaceous (Saccardian taxonomy) fungi. According to the morphology of the parasitic stage, and not according the saprophytic one, it is to be classified as an asporogenous yeast.

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[1]) The term evolution, as applied to fungi, is in any way ambiguous. In most cases, for Protista, "more evoluted" is synonym with "more complicated" in structure or life-cycle.

Only taking in consideration both morphologies, we established, for this genus, an independent family. The same criterium has been applied to the most important genera of fungi pathogenic for man and animals.

As conclusion, we recently proposed (CIFERRI, 1958) a classification based on the presence of different kinds of thallospores and conidia (possibly also endospores, chlamydospores and sclerotia) in the parasitic and saprophytic life-cycle stages.

Particular attention has been paid to the thallospores, and conidia, because they are the most varied and widespread. The following categories were considered:

A) Vegetative mycelial spores (thallospores).
   1) Blastospores, yeast-like, budding cells (asporogenous yeasts and so on).
   2) Aleuriospores (or aleuriae) stalked, as a rule large thallospores, with a not caduceous, narrower aleuriophore, typically acrogenous and single (*Scedosporium* = *Monosporium*; *Emmonsia* = *Haplosporangium*; etc.).
   3) Arthrospores or thallospores produced by fission of mother hyphae (*Geotrichum*; *Hormodendrum*, etc.).
   4) Radulaspores or small thallospores inserted along not specialized hyphae, through small sterigmata-like outgrowth (*Aureobasidium* = *Pullularia*; *Rhinotrichum* = *Sporotrichum*, and so on).
   5) [Chlamydospores or intercalary or apical, polymorphic, short cells, as a rule having a thick epispore (frequent in culture of many genera of fungi)].

B) Conidial spores (or conidia) produced by specialized reproductive hyphae (conidiophores).
   1) Phialospores (or phialidiospores) produced into specialized single cells or phialidia.
      a) Phialomeristem spores produced in basipetal succession into ampulliform phialidia (e.g. *Aspergillus*, etc.).
      b) Endoconidia (or endoconidiospores) produced into and ejected from tubular or cylindric phialidia (*Malbranchea*).
      c) Hemiconidia (or hemispores) produced into an elongate, not inflated phialidium, and isolated with a

fragment of the mother tube (*Sporendonema* = *Hemispora*) [1]).

d) Usual, acrogenous conidia (e.g. *Cephalosporium*, etc.).

Our tentative is necessarily incomplete, because: a) only a few papers have been published along the VUILLEMIN-MASON-HUGHES-TUBAKI line of work, so that the knowledge on the genesis, evolution (and significance) of the thallospores and conidia is still little known; b) the presence in nature of intermediate kinds of thallospores and conidia, forming a bridge between the established categories. For the sake of easiness, we considered in our classification certain morphology as pertaining to one of the recognized morphology, even if not completely typical. It is probable that, in the future, the number of categories of these spores should be increased; c) not all the species of pathogenic fungi are completely clarified in all stages of their life-cycle: there is still some discussion on the structure and origin of certain types of reproductive elements.

The presence of different kinds of thallospores and conidia in living tissues or routine culture is summarized in the following key and table:

ANALYTICAL KEY OF THE FAMILIES OF PATHOGENIC FUNGI [2])

A_1) Reproduction by some kind of spores, with or without mycelium.
  B_1) Cultural dimorphism with blastospores in tissue or cultural conditions approaching parasitic life.
    C_1) With ectospores formed by a peculiar endo-exosporulation Paracoccidioidaceae
    C_2) Without ectospores
      D_1) With aleuriospores .... Histoplasmaceae
      D_2) With radulaspores .... Rhinocladiaceae [3])
  B_2) Without cultural dimorphism.
    E_1) With endospores.
      F_1) With stromatic, fungous tissue; true ascospores in asci Piedraiaceae
      F_2) Without stroma; sporangiospores (or protascospores).
        G_1) Aplanospores (or protascospores) without mycelium in tissue .... Coccidioidaceae
        G_2) Sporangiospores with mycelium in tissue Mucoraceae

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[1]) Sexual or true spores (e.g. in the genus *Piedraia*, *Allescheria*, *Debaryomyces*) and *Coccidioides*, if considered as a member of the Protascomycetes, are included only in the key of families.

[2]) Only the most important families of fungi are included, Actinomycetales excluded.

[3]) Sporotrichaceae, if *Sporotrichum* is accepted instead of *Rhinotrichum*.

E_2) Without endospores.
  H_1) By conidiospores with or without thallospores.
    I_1) By phialospores.
      J_1) By phialospores alone .... Sporendonemaceae
      J_2) By phialomeristemospores (and eventually blastospores) .... Aspergillaceae
      J_3) By phialomeristemospores, radulaspores and blastospores .... Phialophoraceae
  H_2) By thallospores
    K_1) By blastospores.
      L_1) By blastospores alone .... Torulopsidaceae
      L_2) Blastospores and arthrospores Trichosporonaceae
      L_3) By blastospores and radulaspores Aureobasidiaceae
  H_3) By arthrospores
      M_1) By arthrospores alone ...... Geotrichaceae
      M_2) By arthrospores and aleuriospores Trichophytonaceae
  H_4) By aleuriospores alone .... Aleurismaceae
A_2) Reproduction by mycelium alone ..... Madurellaceae ¹)

GENERA AND TYPE SPECIES OF THE FAMILIES

I) Fam. PARACOCCIDIOIDACEAE RED. et CIF.,

Mem. R. Accad. d'Italia, Cl. Sci. Fis. Mat. Nat., vol. VIII (12), pag. 595 (1937).

Pertaining to the Imperfect Entomophthorales. Only the genus *Paracoccidioides* ALM., 1930; type *P. brasiliensis* (SPLEND.) ALMEIDA, 1930, with two subgenera:

a) subg. *Eu-Paracoccidioides* CIF. (1958) for the above quoted *P.* (*Eu-Paracoccidioides*) *brasiliensis*, agent of the South American Blastomycosis, based on *Oidium brasiliensis* SPLEND., 1912;

b) subg. *Loboa* (CIF., 1956, as genus) CIF., 1958, as subgen., with *P.* (*Loboa*) *loboi* (FONS. et ARÊA LEÃO) ALM. et LACAZ, 1949, based on *Glenosporella loboi* FONS. et ARÊA LEÃO, 1940, for the Amazonian, Cheloid Blastomycosis.

II) Fam. HISTOPLASMACEAE RED. et CIF.,

Boll. Sez. Ital. Soc. Int. Microbiol., vol. VI (10), pag. 378 (1934).

A genus near both to the asporogenous yeasts and the moniliaceous *Sepedonium* LINK and allied genera. Type genus: *Histoplasma*

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¹) If at least the type species of the genus *Madurella* possess aleuries and phialospores, the characteristic of the Madurellaceae family must be changed and the genus attributed to the group J.

PROSPECT OF THE FAMILIES WITH CATEGORIES OF SPORES IN TISSUE AND IN CULTURE

Cultural dimorphism	ENDO-SPORES	ECTO-SPORES	CONIDIOSPORES		THALLOSPORES				MY-CELIUM	FAMILIES
			Phialospores	Phialo-meriste-mosphores	Blastospores	Arthrospores	Aleurospores	Radulaspores		
+	TC 1)				TC 1)				C	Paracoccidioidaceae
+					TC 1)		C		C	Histoplasmaceae
+ 1)					TC 1)			C	(T) 8)C	Rhinocladiaceae
	T 2)								C	Piedraiaceae
	T 3)								C 6)	Coccidioidaceae
	TC 4)				C 5)				TC	Mucoraceae
			TC						TC	Sporendonemaceae
				TC	C 5)				TC	Aspergillaceae
			C		TC				TC	Phialophoraceae
					TC				TC 7)	Torulopsidaceae
					TC	TC			TC	Trichosporonaceae
					TC			(T)C	TC	Aureobasidiaceae
						TC			TC	Geotrichaceae
						T(C) 5)	TC	(T) 5)C	TC	Trichophytonaceae
	C 5)					TC			TC	Aleurismaceae
			C?						(T) 8)C	Madurellaceae

T = parasitic stage (tissue); C = saprophytic stage (laboratory routine culture).

1) Under certain conditions; 2) Ascospores; 3) Sporangiospores or protascospores; 4) Sporangiospores; seldom also zygospores; 5) Under certain conditions and for certain species; 6) With exception of saprophytic life or tissues; 7) According the genus; 8) As a rule as sclerotia or allied bodies; but see the footnote 3) of the key.

DARL., 1906; type species: *H. capsulatum* DARL., 1906, comprising two subspecies: 1) *H. capsulatum* subsp. *capsulatum* CIF., 1958, for the agent of the American Histoplasmosis or Darling disease; 2) *H. capsulatum* subsp. *duboisii* (VANBR.) CIF., 1958, based on *H. duboisii* VANBR., 1952, agent of the African, Chronic Histoplasmosis.

A second species of the same genus is *H. farciminosus* (RIV. et MICELL.) CIF. et RED., 1935, based on *Cryptococcus farciminosus* RIV. et MICELL., 1883, agent of the Farcinic Lymphangitis of the Solipeds.

The attribution of a second genus, *Gilchristia* CIF. et RED., 1935, emend. CIF., 1958, to this family is quite probable, but more informations are needed. *Gilchristia* is a generic name substituting the traditional *Blastomyces* GILCHR. et STOKES, 1898, a name duplicating the previous *Blastomyces* COST. et ROLL., 1888, the last one being a genus of saprophytic Moniliales without any relation with the agent of the North American Blastomycosis: *G. dermatitidis* (GILCHR. et STOKES) CIF. et RED., 1934, based on *Blastomyces dermatitidis* GILCHR. et STOKES, 1898.

III) Fam. RHINOCLADIACEAE NANNIZZI,

Repert. Sistem. Miceti Uomo Anim., pag. 440 (1934).

With the genus *Rhinocladium* SACC. et MARCH., 1885. This generic name is accepted instead of the usual *Sporotrichum*, because *Sporotrichum* MATR. et RAM., 1905 for the medical species, appears not to be identic with the older *Sporotrichum* LINK, 1809, for saprophytic species. Only one true pathogenic species (and one or two accidentally pathogenic): *R. schencki* (HEKT. et PERK.) VERDUN et MANDOUL, 1924, based on *Sporothrix schencki* HEKT. et PERK., 1900. The common binomial for this species in America is *Sporotrichum schencki* (HEKT. et PERK.) BEURM. et GOUG., 1906, and in Europe *Sporotrichum beurmanni* MATR. et RAM., 1905. Agent of the Sporotrichosis.

IV) Fam. PIEDRAIACEAE VIÉGAS,

Bragantia, vol. III, pag. 40 (1943), confirmed by CIFERRI, BATISTA et CAMPOS, Publ. No. 45 of the Inst. of Mycol., Univ. of Recife, pag. 1—9 (1956).

This family is pertaining to the stromatic, primitive Ascomycetes, Order Myriangiales, and it is the only fungus producing true and typical asci and ascospores on the human body, as ectoparasite of the ears.

The only genus is *Piedraia* FONS. et ARÊA LEÃO, 1928, probably only with the cheratinophilous *P. hortai* (BRUMPT) FONS. et ARÊA LEÃO, 1928, agent of the Black Piedra, based on *Trichosporon hortai* BRUMPT, 1931.

V) Fam. COCCIDIOIDACEAE CIFERRI,

Arch. Protistenk., vol. LXXVIII, pag. 258 (1932), antedating MOORE, Medical Mycology, pag. 147 (1935).

This genus may be considered as member of the aplanetic Phycomycetes or as a primitive Ascomycetes (Protoascomycetes), near the order Protomycales.

The type genus is *Coccidioides* STILES apud RIXF. et GILCHR., 1896, which type species is *C. immitis* STILES apud RIXF. et GILCHR., 1896, for the agent of Coccidioidal Granuloma.

Usually another genus is considered as pertaining to this family, namely: *Rhinosporidium* MINCH. et FANTH., 1905, monotypic, with *R. seeberi* (WERNICKE) SEEBER, 1912, based on *Coccidium seeberi* WERNICKE, 1900, agent of the Rhinosporidial granuloma. Possibly the very analogy between *Coccidioides* and *Rhinosporidium* is apparent, and the similarity caused by the convergence, both genera being unrelated. In fact, up to now, it has not been possible to cultivate *Rhinosporidium*, nor to reproduce the tissue outgrowth by inoculation of infected tissues.

VI) Fam. MUCORACEAE DE BARY,

Beitr. Morph. Physiol. Pilze, vol. II, pag. 12 (1866) (with different desinence), emend. SCHROETER, Krypt-Fl. Schlesien, vol. I, pag. 148 (1897), nec non VAN TIEGHEM et LE MONNIER, Ann. Sci. Nat., Bot., Sér. 5, vol. XVII, pag. 261—399, 1873; ibid., Sér. 6, vol. I, pag. 41 (1875).

A classical family of Zygomycetes. Several species of the genera *Absidia*, *Mucor* and *Rhizopus* may be parasitic to man and animals.

VII) Fam. SPORENDONEMACEAE OUDEMANS,

Meded. K. Akad. Wetensch., Afd. Naturk., 3 Reeks, Deel II, pag. 115 (1885), emend. RED. et CIF., Atti Ist. Bot. R. Univ. Pavia, Ser. 4, vol. V, pag. 145—148 (1934).

Only the genus *Sporendonema* OUDEM., 1885 emend. RED. et CIF., 1934 (non DESM., 1827, monotypic), with *S. epizoum* (CORDA) CIF. et RED., 1934, based on *Torula epizoa* CORDA (or *S. sebi* FRIES, 1832, if the Friesan starting point is accepted), not rare in Europe, but unusual (and only saprophytic) in America. Mild pathogenic, causing sporotrichoid lesions.

VIII) Fam. ASPERGILLACEAE SACCARDO,

Syll. Fung., vol. IV, pag. 64 (1886).

Well known group of fungi, with several *Aspergillus* species and allied genera more or less pathogenic to man and animals. A peculiar genus of degraded or regressed fungi of this family is *Redaellia* CIF., 1930, isolated from human skin, but not pathogenic. The last named genus is linking phialidial and blastosporic fungi.

IX) Fam. PHIALOPHORACEAE Dodge,

Medical Mycology, pag. 833 (1935) (with different desinence).

Only the type genus *Phialophora* Thaxter (1915) including a few species, saprophytic and/or pathogenic to man, causing Chromomycosis (Chromoblastomycosis). This group of fungi appears to be allied to the imperfect stages of sooty-molds (Capnodiales Imperfecti or Asbolysiaceae).

X) Fam. TORULOPSIDACEAE [1]) Ciferri,

Atti Ist. Bot. R. Univ. Pavia, Ser. 3, vol. II, pag. 143 (1925).

Including the asporogenous yeasts, of which the most interesting genera are the parasitic *Malassezia* Baill., 1889 (= *Pityrosporum* Sab., 1895), agent of the Pityriasis Versicolor, type *M. furfur* (Rob.) Baill., 1889, based on *Microsporon furfur* Rob., 1853. The genus *Candida* Berkh., 1923 (= *Monilia* in medical sense), type *C. albicans* (Rob.) Berkh., 1923, based on *Oidium albicans* Rob., 1853 the agent of thrush, etc. A few other species of *Candida* are more or less pathogenic to man and animals. The above quoted genus *Cryptococcus* Kuetzing, 1833, emend. Vuill., 1901, is the well known agent of meningial lesions; it is based on *C. neoformans* (Sanf.) Vuill., 1901, basonym *Saccharomyces neoformans* Sanfelice, 1895. Ciferri, 1959, made the combination *Lipomyces neoformans* (Sanf.) Cif.

Doubtful is the pathogenicity of species of the genus *Torulopsis* Berl., emend. Cif., 1925, based on *T. rosea* Berl., 1895, now corresponding to *T. pulcherrima* (Lindn.) Sacc., 1906, based on the later described *Torula pulcherrima* Lindn., 1901.

XI) Fam. TRICHOSPORONACEAE Nannizzi,

Rep. Sist. Mic. Uomo Anim., pag. 285 (1934) (with different desinence), emend. Cif. et Red., Arch. Mikrobiol., vol. VI, pag. 69 (1935).

A genus linking blastosporic (Torulopsidaceae) and arthrosporic (Geotrichaceae) fungi. Probably only the genus *Trichosporon* Behrend, 1890, at least in part corrresponding to asporogenous stage of the sporogenous yeasts *Magnusiomyces* Zender, 1925, a segregate from *Endomyces*. Here is comprised the agent of the White Piedra of Ears, caused by *Trichosporon beigeli* (Rabh.) Vuill., 1902, based on *Pleurococcus beigelii* (Rabh.) Rabenhorst, 1867, and a few other, doubtful mildly pathogenic species.

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[1]) This family is frequently known as Cryptococcaceae Kuetzing, 1832 (with different desinence); but *Cryptococcus* Kuetz., 1837, emend. Rivolta, 1863, sensu Vuill., 1901, is not properly selected as type genus, because the different interpretation from Kuetzing up to now. In addition the morphology of *Cryptococcus* species is unusual, among the asporogenous yeasts (not to mention the uncertain ascosporic stage).

XII) Fam. AUREOBASIDIACEAE Ciferri,

Man. Micol. Medica, vol. I, pag. 178 (1958).

The type genus is *Aureobasidium* Viala et Boyer, 1891, based on *A. pullulans* (De Bary) Arnaud, 1910; basonym *Dematium pullulans* De Bary, 1868. The genus has been emended by Grigoraki, 1927. *Cladosporium wernecki* Par. Horta, 1921, causing the Tinea Nigra Palmaris, is but a strain of the common, mostly saprophytic *A. pullulans*.

XIII) Fam. GEOTRICHACEAE Ciferri,

Key Asporog. Yeasts, pag. 9, (1950) (Mimeogr. ed.); An. Soc. Biol. Pernambuco, vol. XIII (2), pag. 140 (1956).

The type genus is *Geotrichum* Link, 1809; type species *G. candidum* Link, 1809, not rarely found in man and animals but not or scarcely pathogenic. In the medical literature it appears mostly as *Oidium lactis* Fres., 1852.

XIV) Fam. TRICHOPHYTONACEAE Vuillemin,

Champ. Paras. Mycoses Homme, pag. 142 (1931) (with different desinence).

This family of well known agents of skin and hair parasites, also called Dermatophytae or Dermatomycetes, includes three genera: *Epidermophyton* Sab., 1910, with only one species: *E. floccosum* (Harz) Lang. et Milochev., 1930; *Microsporon* Gruby, 1843, with several species, type *M. audouini* Gruby, 1843; *Trichophyton* Malmsten, 1848 with many species, type *T. tonsurans* Malmsten, 1848. A few saprophytic genera are pertaining to this family.

XV) Fam. ALEURISMACEAE Vuillemin,

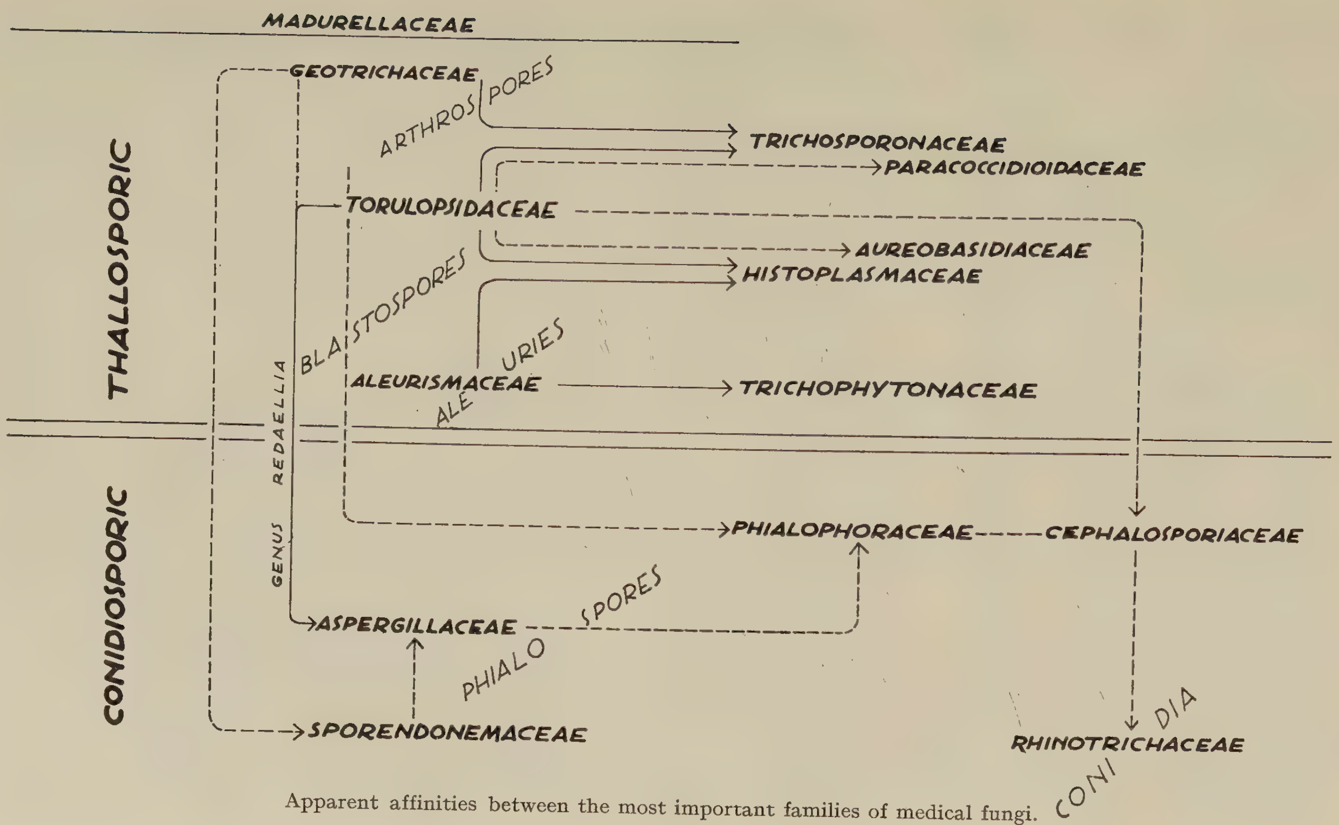
Bull. Soc. Sci. Nancy, Sér. 3, vol. XII, pag. 151, (1911) (with different desinence).

While the type genus is *Aleurisma* Link, 1809 emend. Chevallier 1836, the most representative genus among human fungi is *Scedosporium* Sacc., 1914, type *S. apiospermum* Sacc., 1914, better known as *Monosporium apiospermum* Sacc., 1914, conidial stage of *Allescheria boydii* Shear, 1921, one of the agents of the so-called Madura Foot.

Another genus pertaining to this family is *Emmonsia* Cif., 1959, for *Haplosporangium* Emmons, 1942, non Thaxt., 1914, for *E. parva* (Emm.) Cif., 1959, based on *Haplosporangium parvum* Emm., 1942, mildly pathogenic to animals.

XVI) Fam. MADURELLACEAE Vuillemin,

Champ. Paras. Mycoses Homme, pag. 544 (1931).


MADURELLACEAE
THALLOSPORIC
CONIDIOSPORIC
GEOTRICHACEAE
ARTHROSPORES
TRICHOSPORONACEAE
PARACOCCIDIOIDACEAE
TORULOPSIDACEAE
AUREOBASIDIACEAE
HISTOPLASMACEAE
BLASTOSPORES
ALEURISMACEAE
ALEURIES
TRICHOPHYTONACEAE
GENUS REDAELLIA
PHIALOPHORACEAE
CEPHALOSPORIACEAE
ASPERGILLACEAE
PHIALOSPORES
SPORENDONEMACEAE
RHINOTRICHACEAE
CONIDIA

Apparent affinities between the most important families of medical fungi.

A genus of Agonomycetes or Mycelia Sterilia, with mycelium and sclerotia only (but at least one species of *Madurella* may possess also phialidia and phialospores. In this case this family would be allied to Phialophoraceae). The medical genus is *Madurella* BRUMPT, 1905, with a few species pathogenic to man and causing Madura Foot. Type species *M. mycetomi* (LAV.) BRUMPT, 1905, based on *Streptothrix mycetomi* LAVERAN, 1902, a doubtful species.

Other families of Imperfect Fungi (e.g. Cephalosporiaceae SACC. with *Cephalosporium* and allied genera; Chalaraceae SACC. with *Malbranchea*, and so on) are less interesting to our purpose.

In conclusion, a tentative graphical prospect, schematizing the apparent affinity between the most important families of medical fungi, is appended. It is based on the vegetative, reproductive kinds of spores.

SUMMARY

A key and a plan of tentative classification of the fundamental families of medical fungi are given. It is based on the presence, in tissues or cultures, of different categories of true spores, conidia and vegetative spores (thallospores). The most important "medical" genera of fungi, with type species, basonym, author and data for each family of fungi are listed. The apparent morphological affinity between certain families of fungi is suggested.

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CANDIDA COMPLICATIONS AFTER ANTIBIOTIC THERAPY

by

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(27.X.1960)

Although antibiotics are very widely used and their application for curative and preventive purposes yields beautiful results, medicine today also knows complications that ensue after antibiotic chemotherapy. However, we do not possess any exact information on the incidence of antibiotic therapy failures and complications; the reported data are of casual character.

Failures and "shadowy sides" of antibiotic therapy are extensively described in the literature on the subject. Today we have to deal already with quite a number of facts that call for a critical analysis and correct conclusions.

The monograph written by P. GRATSIANSKY (Paris, 1956) is devoted to the issue of yeast complications after antibiotic therapy and provides an extensive analysis of the author's personal experience as well as that of other authors. Unfortunately, the scientific and practical materials accumulated by Soviet authors were not mentioned in GRATSIANSKY's work altogether. In this connection we could quote here quite a number of names, such as: A. N. ARAVIISKY, A. M. ARIEVICH et al., G. I. BURCHINSKY et al., T. K. GUNDOBINA, G. I. ZAITSEVA, Z. V. ERMOLIEVA, N. P. ELINOVA, F. G. MARCHENKOVA and T. M. KOKUSHINA, A. A. KONDRATYEVA, B. M. PROZOROVSKY et al., and finally our own data published within the past few years (P. N. KASHKIN et al., 1955—1958, V. Y. NEKACHALOV, 1957, etc.).

Failures and complications after antibiotic therapy, as they were decribed in the literature, included toxic and allergic reactions, dysbacteriosis phenomena and infectious processes and side effects connected with it (staphylococcal, protein, pyocyanotic, etc.), impairments of the vitamine exchange in the organism, and, finally, various *Candida*-induced diseases.

The failures of antibiotic therapy are largely accounted for by the irrational application of antibiotics, without due consideration of the causative organisms' response to these drugs, also by the appearance of antibiotic-resistent and sometimes even antibiotic-induced microorganisms in the organism of the patient, by the inadequacy of specific protective reactions of the organism even in cases when antibiotics exert direct action on the causative organisms of the disease.

Prolonged course of disease, its transition into chronic form, the appearance of relapses in certain cases and reccurrence of disease are the most essential and troublesome disadvantages of antibiotic therapy.

The pathogenesis of complications after antibiotic therapy, is, we think, quite multifarious and therefore brooks no exact definition. The appearance of toxic and allergic complications largely depends on the state of the organism, the tolerance of antibiotics by the patients, their sensitivity to them or individual reactions of patients to the mode of administering antibiotics.

The local application of antibiotics, which in some cases leads to the preliminary sensitization of the organism, may sometimes be accompanied by allergic and even anaphylactic reactions, various skin lesions including the so-called contact dermatitis in those people who systematically handle antibiotics in the respective industry, in medical practice, drug stores or laboratories.

The appearance of dysbacterioses, impairments of vitamine exchange and the development of secondary infections, *Candida* infection for one, are to a great degree caused by the prolonged parenteral administration of antibiotics and the shifts in the microbial associations of human mucous membranes connected with it. The role of the patients' individuality in this group of complications can hardly be considered important, speaking in relative terms, of course.

The complexity of pathogenesis of antibiotic therapy failures is connected, one should think, with a combination of factors leading to complications, the interrelations of these factors and conditions which appear in the course of disease, with the "chain" character of pathological alterations. Therefore, a belated disclosure of antibiotic therapy complications and failures, especially if it was prolonged, demonstrates a multiplicity of pathological phenomena in patients which seem different at a first glance although actually they ensue from one another and are instrumental in bringing about new alterations in the organism. Thus, a prolonged use of antibiotics leads to the development of antibiotic-resistent microbial forms instrumental in the appearance of dysbacteriosis. It impairs the production of vitamines in the organism and that, in turn, leads finally to hypo- and avitaminosis, creates conditions favourable for the development of *Candida*-caused and other secondary and infectious diseases. The activity of hypothetically pathogenic and putrefactive microorganisms increases against the background of dysbacteriosis, septico-pyemic and toxic processes get under way which gradually weaken and decrease the protective reactions of the organism, etc.

In this connection diseases caused by the fungi *Candida* drew the attention of investigators. The *Candida* diseases were, of course, known prior to the invention of antibiotics and the attitudes of scientists towards them were quite different. Diseases caused by the fungi *Candida* became more frequent in the period of extensive use of antibiotics. They are studied now in more detail than previously, and the evaluations of such diseases by various scientists differ greatly. The opinion of PARROT who denied the independent etiological role of the thrush fungus already in the last century is reflected in the skepticism displayed today by T. BENEDEK in respect to the fungi *Candida* in general.

Other authors, although they do not deny the involvement of microorganisms in the pathological process which develops in some cases of antibiotic therapy are inclined to consider the fungous infection in such cases as one of manifestations of "drug disease" (E. M. TAREEV) or the intolerance reactions in the spirit of HURIER's concepts (Cl. HURIER, 1954).

In this paper we can but briefly outline the main characteristics of *Candida* complications in the course of antibiotic therapy. This topic was the subject of combined investigations carried out between 1953 and 1958 in the Clinics and Laboratories of the Leningrad Institute of Antibiotics and the Leningrad Institute for the Advanced Training of Physicians.

On the basis of clinical and laboratory observations carried out in 200 cases of suspected secondary *Candida* infection we ventured on providing the clinical characteristics of *Candida* complications, the assessment of laboratory investigations and criteria used in elucidating the etiological role of the yeastlike fungi in the development of complications after an unsuccessful course of antibiotic therapy.

We deem it also advisable to dwell on some pathogenic premises involved in the appearance of *Candida* complications and also on the methods of treatment and perspectives of prophylaxis of such complications.

Those diseases were considered by us to be caused by the fungi *Candida* which had the proper clinical picture and when we constantly detected the presence of the yeastlike fungi of the genus *Candida* — the fungi which are the most probable causative organisms of the disease. This last statement was confirmed by investigations carried out dynamically with due consideration of an increasing number of the fungi colonies in the affected areas, intensification of serological reactions (agglutination and complement fixation reactions) and well pronounced allergic tests of *Candida*-antigens (vaccines, extractions) and also the success of antimycotic therapy.

Those conditions were suspected of being *Candida*- induced which had no clearly outlined clinical symptomatology and lacked the support of the above-mentioned data of laboratory investigations.

Such forms were sometimes observed at the earliest stages of the disease which much later assumed the characteristic traits of a well pronounced *Candida* process.

The initial forms of *Candida* complications were the most difficult to diagnose since they lack the clearly manifest clinical picture. It was especially true of the long-term carriers of the yeastlike fungi on the skin and mucous membranes.

Single findings of the yeastlike fungi of the genus *Candida* on the mucous membrane of the mouth and on the skin, in the phlegm, urine, even pus which were not substantiated by clear specific clinical manifestations or sufficiently good results of serological reactions sometimes performed repeatedly and in dynamics — such findings were not considered by us as irrefutable proof of the *Candida* nature of the main disease.

We had reasons to believe as definite *Candida* complications after antibiotic therapy such diseases which, having occurred in connection with antibiotic therapy, disappeared after discontinuation of treatment with an ineffective antibiotic, or after eliminating the pathogenic premises instrumental in the development of *Candida* complications (dysbacteriosis, hypo- and avitaminosis, etc.).

The clinical picture and course of antibiotic therapy *Candida* complications were extensively variable: form superficial to profound and wide spread visceral lesions; form a shortlived to a prolonged and chronic process; from a mild to an extremely grave course of the disease sometimes ending fatally.

At the beginning of our study (1953) the grave forms of *Candida* diseases were more frequent in our practice. This can be explained, we believe, by the late disclosure of the real nature of these complications, generalization of infection, septic manifestation and especially because in some cases the cause of infection was revealed only at the post-mortem examinations. In later years serious forms of the disease were observed less frequently due to the most extensive health education campaign launched, the aim of which was to provide information on the causes of failures and complications of antibiotic therapy. Lethal outcome was reduced almost to nil and was observed only in those cases in which the patients were of old age or where the disease progressed against the backgrounds of general emaciation of the organism and weakening of protective reactions, also against the background of other diseases with lethal outcome.

Concerning the clinical manifestations of the disease, the most frequent ones were the following: the *Candida* affection of the skin and the mucous membranes, less frequently the lesions of the internal organs and very rarely the septico-pyemic forms of the disease. Besides, there were atypical and insufficiently clear pictures of the disease. The appearance of the latter, although connected with the administration of antibiotics, could not be considered as genuine *Candida* processes due to their character (even in the presence of the yeastlike fungi in the affected area and positive allergic reactions). Among the above manifestations the following deserve attention: hypertrophy of the lingual papilla, the black tongue (glossotrichia), erythematous tongue, the so-called "mycotic aphthoids", pseudopustulous stomatitis and some other complications.

Finally, we can not help pointing out the specific group of diseases affecting the skin and the mucous membranes where the administration of antibiotics is accompanied by an intensified development of the yeastlike fungi which complicate other diseases both of mycotic and non-mycotic nature with their metabolic products. Here belong: dysidrosis, pyodermia, microbial eczema, pemphigus, psoriasis, erythrodermias, acute lupus erythematosus and many others.

The above conditions do not include cases observed in the course of antibiotic therapy in which the yeastlike fungi were detected, sometimes in increasing quantities, although there were no convincing results of serological tests or of clinical picture characteristic of *Candida* affections. The number of such cases amounted to 1,000.

Clinical forms of *Candida*-caused diseases are shown in Table I.

The *Candida*-induced skin complications and the mucous membrane affections were quite multifarious. They were: 1) superficial ulceration and erosion on the mucous membrane of the oral cavity and tonsils, affections resembling thrush in adults and children; 2) intertriginous affections of the major and minor skin folds with the ensuing lesions of the body skin; 3) bronchopneumonia of an interstitial character taking a long course with relapses and non-responsive to either antibiotic or sulfonamide therapy; 4) complications of the type of peculiar chronic sepsis, septic endocarditis; 5) cystitis and pyelocystitis, the increasing amount of the yeastlike fungi being detected in the aseptically treated urine. Here also chemotherapy was of no avail; 6) diarrhea and other dyspeptic disorders without the presence of any pathogenic or specific microorganisms that could account for the cause of the disease; 7) postinfectious intoxications observed at the period of disappearance of the causative organisms of the main disease (e.g. in cases of dysentery, pneumonia) and reappearing against the background of intensive antibiotic therapy. All attempts of administering antibiotics invariably aggravated the condition of the patients.

An analysis of *Candida*-induced complications with due regard to the main disease demonstrated that the overwhelming majority of cases observed by us suffered from pneumonia and dysentery, less frequently from septic conditions, endocarditis, and, in decreasing sequence — from cystitis, and pyelonephritis, tonsillitis, malignant tumors of the lungs and other diseases; the abovementioned diseases were rather often accompanied by dyspepsia and hypovitaminosis.

Table I.

Candida Complications Observed in the Course of Antibiotic Therapy

1. Skin affections	70	cases
among them:		
a) eruptions of the type of "yeast eczematoids" and other skin eruptions	33	,,
b) aggravation of paronychias	8	,,
c) perlèche (without other clinically determined affections of the skin and mucous membranes)	5	,,
d) aggravation of pemphigus and During's dermatitis accompanied by the *Candida* affection of oral mucous membranes	19	,,
e) skin affections in cases of acute lupus erythematosus (aggravation of main process with *Candida* infection)	5	,,
2. Affection of mucous membranes	38	cases
among them:		
a) oral affections (cheilitis, stomatitis)	8	,,
b) pharyngeal affections (tonsilitis)	8	,,
c) affections of genitalia	5	,,
d) ulcerative-aphthous symptom—complex	7	,,
3. Affection of respiratory organs	52	cases
among them:		
a) pulmonary complications of the type of "nonspecific" pneumonias with relapses and aggravations in the course of antibiotic therapy	48	,,
b) asthmatoid symptom—complex	4	,,
4. Manifestations of "candidosepsis"	6	cases
5. Intestinal affections	3	,,
6. Cholecystitis	8	,,
7. Urological complications (pyelo-cystitis)	3	,,
8. Blepharo-conjuctivitis of yeast etiology	4	,,
9. Postoperative Candida complications of types	17	,,
10. Other unclear Candida processes (vascular affections, endocarditis, lymphogranulomatosis)	11	,,
TOTAL NUMBER OF CASES	202	

The *Candida*-induced complications were most frequently observed in patients continuously treated with antibiotics either one at a time or in combination according to the following drugs respectively: 1) penicillin, streptomycin, cyntomycin, levomycitin (aureomycin), biomycin (chlortetracyclin); 2) penicillin, streptomycin, cintomycin and biomycin; 3) penicillin, biomycin, streptomycin; 4) penicillin, biomycin, albomycin.

Complications were not as frequent when only one drug was used at a time, penicillin or streptomycin or in combination, the therapy being very long sometimes. The dosage of separate antibiotics was always high although they were administered in combination with the others. In some cases tens and even hundreds of millions of units were administered.

Candida-induced complications developed irrespective of the mode and route of administration of antibiotics. The attempts to establish the direct connection between the complications and the mode and route of administering this or that antibiotic brought no positive results, although it can be pointed out that, of recent years, complications are rather more frequent when antibiotics are administered parenterally. This route usually calls for an increased amount of the drug to be administered, and, besides, there is a definite meaning in the direct effect of antibiotics on the highly sensitive normal microflora of the mouth, pharynx and intestines.

This creates conditions for a rapid development of dysbacteriosis and alterations of the mucous membranes.

We found the following common features of *Candida*-induced complications: a) direct connection with large doses of antibiotics, sometimes administered as a combination of 3—5 drugs; b) hypo- and avitaminosis; c) emaciation of the organism as a result of some recent grave disease or infection or in the process of some chronic serious disorder; d) the predominant affection of infants and old people suffering from various excruciating diseases accompanied by the disorders of metabolism, hormonal impairments, etc.

The clinical course and the outcome of the *Candida*-induced diseases were rather diversified. Grave forms of the disease and lethal outcome were observed almost exclusively in very young children. Serious cases among adults were encountered as a rule after a long and ineffective administration of antibiotics of a broad spectrum and in very large doses. Lethal outcome was relatively frequent in 1953—1954. It was observed almost in all cases among children after some serious diseases like dysentery, pneumonia, etc., when the real diagnosis was very often established after the post-mortem (cultural and histological) investigation of the material. This is accounted for by the sufficient knowledge of the nature of *Candida*-induced diseases and late disclosure of the mycotic origin of complications.

In later years, as the knowledge of *Candida*-induced diseases expanded and antibiotic therapy was prescribed more carefully, the grave forms of the disease were on the decrease. They could be observed in advanced neglected cases of septic complications among adults and patients emaciated by other diseases.

The three following groups can be differentiated as regards the prognosis and outcome of the *Candida*-induced diseases:

Group I. This is the largest group with the highest incidence of affections which are as follows: mild forms of *Candida*-induced diseases of the mucous membrane of the mouth and genitalia in children and adults. These complications have the most favourable prognosis: they disappear by themselves or after a short administration of common antimycotic drugs, iodine preparations, borax and aniline dyes, etc.

Group II. This group consists of clearly manifest but benign

Candida affections: mouth thrush, pharyngitis and rhinitis, dermatitis of major skin folds and erosio interdigitalis and disquamation, cheilitis, perlèche and tonsillitis, onychia and paronychia, vaginitis, cases of long diarrhea and anal itch.

Group III. This group includes grave *Candida* affections with a tendency toward generalization. They can start from the affection of the mucous membrane of the mouth and skin or from the direct involvment of internal organs into the process (lungs, intestine, etc.).

The *Candida*-induced diseases take an especially grave course in combination with other emaciating diseases both infectious and non-infectious; in spite of energetic antimycotic treatment lethal outcome may easily set in in such cases.

The diversity of clinical manifestations of *Candida* affections, the similarity of many *Candida* affections with other infectious and non-infectious diseases especially in cases when the yeastlike fungi stayed for a long time in the microbial associations on the skin and in the mucous membranes of healthy people and recently recovered cases — all these factors sometimes make it very difficult to diagnose *Candida* diseases. The real origin of *Candida* affections can be disclosed by a combined clinico-laboratory analysis of patients alone.

Without being sure of the *Candida* origin of the disease, it is impossible to diagnose. The interpretation of these forms of the disease becomes more difficult in view of the fact that very often they are considered to be complications after antibiotic therapy.

We are extensively using in our practice the results of microscopic and cultural examinations of fungi as well as the allergic tests and the serological reactions with due regard to the yeastlike fungi isolated from the patient and the pathogenicity test. All these are used as criteria for elucidating the etiological role of the fungi *Candida* besides other clinical manifestations characteristic of *Candida* affections.

Without the necessary data on the intensity of *Candida* affections, without the knowledge of immunological alterations in the human organism, the cultural and serological investigations cannot be considered exhaustive proof of the *Candida* origin of complications taken separately. This is especially true of those conditions where the clinical picture is not clear enough.

It goes without saying that the detection of the yeastlike fungi in closed foci of infection and also in areas usually unaffected by fungi (urine, blood, spinal fluid, pleural exudation, etc.) even when other microorganisms are absent, is weighty enough proof of the *Candida* origin of the disease. Especially demonstrable was the detection of fungi of the genus *Candida* in histological sections of the warty growth of cardiac valves in cases of endocarditis and also in the intestinal wall in children unsuccessfully treated with antibiotics and with lethal outcome after dysentery (the elements of a yeastlike fungus grew through the tissue including the muscular layer).

The quantitative characteristics of the yeastlike fungi through the medium of microscopic studies of pathological material are hardly effective and the results are not always convincing, in our opinion: the final results may differ even after the application of different tests to the same material. This last statement can be explained by the peculiarities of the focal location of the yeastlike fungi in the tissues, the accumulation of their colonies in the phlegm, pus and other substrates. This evidently goes to account for the unusual detection of the yeastlike fungi in different preparations of the same pathological material when studied microscopically. This is frequently the case with the microscopic studies of nail scrapings and skin scales, the matter of ulcers, phlegm, pus, etc.

The disadvantages of detecting the yeastlike fungi in open foci of affected areas, the difficulty of determining them quantitatively, especially when the amount of material under study is not large, render the results of a simplified investigation by means of a microscope unstable and of little value for making final diagnostic conclusions.

The cultural data accompanied by a description of the yeastlike fungi, with due regard of an increasing amount of colonies of the genus *Candida* after repeated inoculations of the material are a very important link in the chain of other proofs of the *Candida* origin of affections which were not clear at the beginning.

We believe it necessary to determine the pathogenicity of isolated cultures in all cases in dealing with yeastlike fungi of the genus *Candida* except for *Candida albicans*. The pathogenic significance of the latter has been established by numerous investigations of the past together with extensive animal experiments and even special injections administered to human beings by some investigators.

The overwhelming majority of strains of *Candida albicans* isolated from human beings are pathogenic for laboratory animals, although the virulence of some strains can vary within a rather broad range.

We attach special significance to the complement fixation test among all serological reactions due to its specificity and stable nature of results. This test has advantages compared with other methods and it occupies a prominent place in the laboratory diagnostics of some forms of *Candida* affections, especially in those cases when the disease is over a year's standing and wide spread.

An increase of antibodies and the intensity of this reaction may be good proof of an increasing activity of the *Candida* fungi in the organism and its etiological significance under specific conditions.

Positive agglutination reactions of the yeastlike fungi of the genus *Candida*, especially in the low titre dilutions of tested sera, do not have (according to our materials) an important diagnostic significance: the agglutinins are detected rather often also in the sera of both the infected persons and in healthy ones. It is but the dynamics of agglutinins and their increase in the process of disease (1 : 120 — 2,560) that can be utilised for the confirmation of the diagnosis or the *Candida* nature of the disease

Positive allergic reactions, especially in the process of their intensification, can also be used for elucidating the *Candida* origin of the diagnosed disease. They are rather permanent in cases of *Candida* affections of the skin and are quite weak in cases of the yeastlike fungi carrying limited affections of the visceral organs and also in some forms of candido-sepsis.

The comparison of results of different methods of laboratory investigation and the parallel study of the clinical picture and course of the disease yield the most interesting results when analysing *Candida* affections with a long clinical course and wide localization of the pathological process — and that even when the general state of the patient's health is satisfactory.

The correspondence between the results of laboratory investigations (complement fixation test, etc.) in cases of well pronounced systemic and visceral *Candida* affections, even without the involvment of the epithelium into the process, is quite a normal phenomenon.

In cases of superficial *Candida* affections, the parallelism between the clinical and laboratory results is less constant and the correlation is less satisfactory.

It is necessary to establish the intensity of immunobiological reactions of the disease in progress with an account taken of the number of the yeastlike fungi in the foci of affection (in the smears, colonies, etc.) and their increase so as to be able to assay the *Candida* origin of the disease.

Clinical observations alone, no matter how clear and convincing they may be, are not sufficient diagnostic material to prove the *Candida* origin of the disease. This our opinion is based on many years of personal experience in the given field. Single findings of the *Candida* fungi in the affected areas in the skin and mucous membrane cannot be considered as final proof of the *Candida* origin of the disease either. The same is true of accidental positive results of serological tests, especially so when the titre of antibodies is low. It is common knowledge that this can be the state of affairs with quite normal individuals and also the carriers of the fungi *Candida* on the mucous membranes and in the skin.

Our own experience of controlling the *Candida*-induced diseases, apart from some details, is nothing new compared with the already known methods. Together with other authors we can say that in many cases *Candida* affections due to antibiotic therapy, disappear after the discontinuation of antibiotics. In other cases common antimycotic drugs relieve the suffering. These are the drugs that are always prescribed in cases of thrush (borax, iodine solutions, aniline dyes).

It was much more difficult to achieve a stable and speedy effect in some peculiar cases of *Candida* infection (chronic affections of the skin and mucous membranes, visceral organs and especially "candido-sepsis"). In such cases the removal of the yeastlike fungi from the patient's organism was a very difficult task. In certain cases when the fungi were totally wiped out from the organism ("Nistatin" therapy and other drugs) still no stable recovery set in. Evidently the alterations that occurred in the organism under the effect of *Candida* were so grave that the restoration of the patients' health could not be achieved by eliminating the fungi *Candida* from the organism alone. Hence the necessity to improve the general state of the organism, to mobilize its protective forces and not only to eliminate the yeastlike fungi of the genus *Candida* from it.

"Nistatin" proved to be the most effective drug in our experience with the *Candida* fungi. We administered the drug in 5-7 day cycles with 7-10 days of intermission between the cycles. The dosage is 4-6 tablets of the drug daily in cases of moderate severity (one tablet contains 5000,000 curative units of the drug). The total average cycle consisted of 28-30 tablets. A decrease or total disapearance of the *Candida* fungi was noted already on the 2-4th day of treatment, although in some cases the fungi were detected even after the total course of therapy was over. The fungi persisted especially in those persons who were suffering from chronic forms of the *Candida* infection. In cases of *Candida*-induced cholecystitis the fungi disappeared only at the end of the third or second cycle of treatment with Nistatin, each cycle consisting of 7-10 days and 8-10 tablets of the drug daily.

In cases of asthma the improvement took some time to set in. In our opinion the cause of asthmatic attacks was evidently in the presence of the yeastlike fungi for a long time in the respiratory passages where they played the role of specific allergenes. Energetic Nistatin therapy led to the recovery of such patients who suffered from asthmatic attacks for years and could not be cured by any other methods. The diagnosis and recommendations in these cases were based on the findings of the yeastlike fungi of the genus *Candida* in the phlegm (in big amounts), the positive complement fixation reaction with candidoantigens and also aggravation of the mouth thrush, especially at the time of administering antibiotics.

However, as was already said, the Nistatin therapy alone was in many cases not sufficient and we therefore, prescribed other drugs used in combination with one another and in strict succession so as to obtain stable results.

Very often positive effect was observed after administering iodine preparations per os and at the same time (5-7-10-day course regardless of tolerance) intravenous injections of 40 % solution of urotropin (3-5-8 ml).

In some cases positive results were achieved by making intravenous injections of potassium thiosulphate, methylene blue and also due to the intravenous injections of ethyl alcohol solution (10-20-30 % solutions, 20-30-50 ml daily in the course of 1½-2 weeks). The last method was used in cases of *Candida* affections of the lungs and manifestations of "candidosepsis".

In cases of affections of the intestinal tract the preparation of the gastric juice by I. P. PAVLOV's method proved very useful, as were as weak solutions of sulphuric magnesia (indicated in cases of the fungi affections of the billiary ducts and intestine) and 0,1 % solution of gentiana violet. A. N. ARAVIISKY observed good results in advanced cases of cheilitis after oral administration of potassium salicylate.

Vitamine B_1 should be used preferably to other vitamines in our opinion. We are inclined to believe that large doses of this vitamine bring the desired effect (5 % solution of Vitamine B_1, 2—3 ml daily intramuscularly).

There are no reasons, in our opinion, for strict limitations in the diet. We do not exclude from the diet sugar and other carbohydrates if they are used in normal quantities. At the same time caution should be observed in administering glucose for curative purposes in cases of visceral and generalized forms of *Candida* affections (intravenous injections, especially in combination with antibiotics). We should like to point out, without laying special stress on a specific diet, that it should be rich in proteins and vitamines (curds and eggs). The diet should also contain Yoghurt and sour milk as well as other milk products.

Transfusions of conserved blood, blood plasma and other hemopreparations can be of great use to patients suffering from diseases caused by the fungi *Candida*.

In spite of the fact that vaccine therapy is not yet totally assessed in cases of *Candida* affections we use this particular type of therapy (when the general condition of patients improves) to increase the specific protective reactions of the organism. We are using for this purpose either "tetravaccines" prepared from the most widely spread species of *Candida*, or "monovaccines" or "autovaccines" prepared from the fungi *Candida* isolated from the patient. These preparations contain 200 million yeastlike cells in 1 ml of suspension killed at the temperature of 70° within one hour.

The vaccines are administered either intracutaneously (0.1—0.2—0.3—0.5—0.8) or intramuscularly (starting from 0.2—0.3 and gradually increasing the dose from 0.5—0.8—1.0—1.5—2.0 of the vaccine per one injection). The average is 8—10 injections per course of treatment made once or twice weekly with due regard to the reactions of the organism and the achieved effect. The first half of the course of vaccine therapy should be administered intracutaneously and the second half — intramuscularly.

The application of insulin in a number of cases resulted in eliminating the manifestations of *Candida* affections not only in patients suffering from diabetis mellitus but also in those who did not have such symptoms.

At the present time it is hardly possible to name any other definite drugs and give recommendations for the treatment of *Candida* affections.

Positive achievements in the therapy of *Candida* conditions are as follows: elimination of clinical manifestations of *Candida* affections and lack of relapses, normalisation of the blood content and erythrocyte sedimentation rate, improvement of the vitamine balance of the organism, increase of body weight of patients, improvement of general condition and increased working capacity. With regard to the laboratory data, the following are the most significant: decreasing number of findings of the yeastlike fungi in the pathological material, a systematic isolation of cultures when inoculating the material under study, etc. Serological and allergic reactions have not been given the final assessment in this respect. No other proof of the efficacy of therapy exist at the present time.

Further study should be performed to elucidate the causes of the gravity of *Candida* processes in some cases, their resistence to therapeutic measures even when combined methods of treatment are used. The role of the functional state of the patient's organism should be given further consideration together with elucidating the role of the central, sympathetic and parasympathetic nervous systems.

The hormonal state of the organism also plays some definite role in the development of *Candida* diseases in general and antibiotic therapy complications in particular.

The search for new antibiotics and other drugs effective in the control of *Candida* infections remains very important for the achievement of positive management in cases of *Candida* affections.

The above statements do not exhaust, of course, all the problems arising out of the curative and preventive use of antibiotics.

"The shadowy sides" of antibiotic therapy registered in some cases do not decrease the colossal role of antibiotics and their powerful action against numerous infections. At the same time it is necessary to study thoroughly the side effects and results of antibiotic therapy, continue to work on the problems of rational and scientifically established use of antibiotics so as to avoid the undesirable complications and negative effects. Concerning the complications we can say that *Candida* affections still remain a part of one big general biological problem the final solution of which is in the future.

SUMMARY

Comparative clinical and laboratory studies were carried out on patients with *Candida* complication due to antibiotic therapy.

Pathological and clinical peculiarities of the causative organism, the mycological and serological diagnoses have been investigated and diverse criteria of a definite *Candida* etiology of the complications have been developed.

As *Candida* complications those afflictions have been considered which developed in connection with the antibiotic therapy and which disappeared after cessation of the same.

The general symptoms of *Candida* complications observed are the following: 1) a direct relation with a prolonged treatment with large doses of antibiotics, often in combination of three to five preparations; 2) the presence of symptoms of a hypo- and/or avitaminosis; 3) the emaciation of the organism due to a severe infection or to a chronic pathological process; 4) a prevalent occurrence in children of early age and in older persons suffering of metabolic or hormonal disturbances.

Candida complication of the skin and the mucous membranes have been the most frequent ones, less frequent were the attacks on internal organs or the septicopyemic forms.

As evidence of the *Candida* etiology of the complications has been considered: an intensive development of yeast-like organism in the affected areas; the presence of antibodies as agglutinins, precipitins, and complement fixing antibodies; the intensity of allergic skin tests during the development of the pathologic processes.

The complement fixation test had a definite advantage compared with the other serologic methods due to its specificity and stability of the results.

In the treatment of *Candida* complications a great number of useless preparations were eliminated and instead, Nystatin, Iodides, vitamins and *Candida* vaccine were used.

The authors conclude that a constant clinical and laboratory control of the effect of antibiotics, an early recognition and treatment of the primary forms of *Candida* complications on the skin and mucous membranes are of great advantage in the prevention of *Candida* complications of an antibiotic therapy.

ZUSAMMENFASSUNG

Es wurden vergleichende klinische und Laboratoriumstudien von Kranken mit kandidösen Komplikationen einer antibiotischen Therapie durchgeführt.

Pathologische Besonderheiten, klinische Eigentümlichkeiten des Erregers, die mykologische und serologische Diagnostik wurden untersucht und verschiedene Kriterien für die Zweifellosigkeit einer kandidösen Natur der Komplikationen ausgearbeitet.

Als kandidöse Komplikation betrachtete man Krankheiten, die sich in Zusammenhang mit der antibiotischen Behandlung entwikkelten und nach Beseitigung der pathogenen Bedingungen, die die Entwicklung kandidöser Erkrankungen begünstigten, verschwanden.

Die allgemeinen Merkmale der festgestellten kandidösen Komplikationen sind die folgenden: 1) ein direkter Zusammenhang

mit einer dauernden summarischen Behandlung mit grossen Dosen von Antibiotika, die öfters in einer Kombination von 3—5 Präparaten gleichzeitig verordnet werden; 2) das Vorhandensein von Symptomen einer Hypo- oder Avitaminose; 3) eine Auszehrung des Organismus infolge einer bestandenen schweren Infektion oder eines chronischen Krankheitsprozesses; 4) ein vorwiegender Befall von Kindern im frühen Alter und von älteren Personen, die an Stoffwechselstörungen, hormonalen Störungen usw. leiden.

Am häufigsten gibt es Haut- und Schleimhautkomplikationen; bedeutend seltener beobachtet man ein Befallensein der inneren Organe und das Auftreten von septisch-pyämischen Formen.

Als Beweis einer kandidösen Natur der Komplikationen galten die Befunde einer intensiven Entwicklung hefeartiger Pilze in den Befallsherden. Antikörper: Agglutinine, Präzipitine und Komplement-bindende, sowie auch die Intensität der allergischen Hautproben während der Krankheitsentwicklung.

Die Reaktion der Komplementbindung hatte kraft ihrer Spezifität und der Stabilität der erhaltenen Resultate einen deutlichen Vorzug vor anderen Methoden der Laboratoriumsdiagnostik von Kandidosen.

In der Behandlung von kandidösen Komplikationen wurde die grosse Bedeutung einer Beseitigung von wertlosen Präparaten, einer Verordnung von Nystatin, Jodiden und Vitaminen und einer Anwendung von Vakzinen aus hefeartigen Pilzen gen. *Candida* festgestellt.

Die Autoren meinen, dass eine ständige klinische und Laboratoriumkontrolle über die Wirksamkeit der Antibiotika, eine möglichst frühe Erkenntnis und Behandlung der primären Formen von kandidösen Erscheinungen an Haut und Schleimhaut für das Vorbeugen kandidöser Komplikationen einer antibiotischen Therapie von grossem Nutzen sind.

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STUDIES ON SOME SPECIES OF *EPHELIS* AND *BALANSIA* OCCURRING IN INDIA

I. Morphological account of some species of *Ephelis*

H. C. Govindu[1]) & M. J. Thirumalachar

(24.X.1960)

(with 17 figs.)

INTRODUCTION

The genus *Balansia* Speg. and its conidial form *Ephelis* Fr. forming an important group of plant parasitic fungi in the Clavicipitoideae, are of widespread occurrence in India on several grasses and cereals. The monograph on "Balansia and the Balansiae in America" by Diehl (1950), though confined to the North and South American species, has brought together very valuable information on the general morphology of the fungus, host relationships, nature of parasitism etc. The importance of the conidial stage in distinguishing the genera of the Clavicipitoideae has been stressed by Diehl, and the genus *Balansia* has been characterised by having *Ephelis* for the conidial stage.

Species of *Ephelis* parasitise graminaceous hosts in India, several of them being of economic importance. *Ephelis oryzae* Syd. on rice (*Oryza sativa* Linn.) for instance, was first described by Sydow (1914) on the basis of the material collected in South India. Ephelidial stages on two grasses, *Eragrostis tenuifolia* Hochst. and *Isachne elegans* Dalz. were referred to *E. oryzae* tentatively by Venkatakrishnaiya (1946). He later listed two more cereals, *Setaria italica* Beauv. and *Echinochloa crus-galli* Beauv. as hosts for *E. oryzae* (1952). Patel et. al. (1951) found a species of *Ephelis* inciting sterility of spikelets in *Aristida* species, near Poona, and referred it to the conidial stage of *Balansia andropogonis* Syd. previously recorded in India on *Andropogon aciculatus* Retz. Govindu & Thirumalachar (1954) reported an *Ephelis* species parasitising the spikelets of an important fodder grass *Sorghum halepense* (Linn.) Pers. in Mysore. Recently (1959) Govinda Rao et. al. reported *Ephelis* on four graminaceous hosts, *Arthraxon ciliaris* var. *coloratus*, *Cynodon dactylon*, *Saccolepis indica* and *Secale cereale*. Based on size of spores, *Ephelis* on all of these four hosts is tentatively referred to *E. oryzae*.

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MATERIALS FOR STUDY

Ephelidial stage parasitising the spikelets and vegetative parts of different grasses and cereals were collected on more than twenty hosts by the authors in different parts of India, chiefly in Bihar and Mysore. Morphological and cultural studies of several of them were undertaken. In the present paper an account of the morphological studies of the fungus is presented.

DISEASE SYMPTOMS

All species of *Ephelis* so far known incite systemic infection of the host, the diseased plants appearing apparently vigorous and similar to the healthy plant until the time of flowering. In few cases, however, the diseased plants are stunted and paler in colour than the healthy ones. Infected plants show a white effuse covering of hyphae on the upper leaf surface, which is very distinct and evident especially in the younger leaves. The name "silver-leaf disease" is often referred to indicate this disease symptom. While most of the *Ephelis* species reported from America are known to produce the *Balansia* stage, data on hand indicate, that many of the *Ephelis* species in the tropical climate like India are able to continue indefinitely in the conidial or mycelial stage alone without forming the ascigerous stage.

While the fungus is systemic within the host and affects all parts of the plant, in few instances like *Dactyloctenium aegyptium* BEAUV., *Oryza sativa, Pennisetum hohenackeri* HOCHST., the diseased clums are often seen to bear a few healthy inflorescence also. This has been found to be due to rapid growth of the host much in advance of the pathogen, resulting in disease escape in certain portions of plant. This feature is further illustrated in cases where the basal portions of the inflorescence show infection and the top portions are free from the disease.

CHARACTERISTICS OF THE FUNGUS

The structure of the mycelium within the host, the types of ephelidial fructifications and conidia have already been described in detail by DIEHL (1950). The fruiting body described to be a patelloid excipula in some species is, however, not very apparent in the Indian collections studied. The appearance is more like an irregular sporodochia, comparable with the Sphacelial stage of *Claviceps*. The conidiophores are delicate, simple or branched, compactly grouped, bearing numerous acicular, hyaline, often gattulate conidia, which are bound up with a viscid matrix to form a greyish-white glutinous mass. The conidial mass is soft and white in the young sori, and later dries up into a hard crust. When a diseased spikelet is mounted in a droplet of water, the glutinous substrata dissolves and releases numerous conidia.

The ephelidial fructification manifests itself in majority of the hosts in the entire inflorescence and/or in the individual spikelets. When the fructification appears on the entire inflorescence, it is recognised at the heading stage by the transformation of all the spikelets into greyish or greenish cylindrical rod. In a few cases, however, the fructification appear on the vegetative parts of the plant, especially on the young top leaves. With the exception of *Brachiaria distachya* STAPF, *Cymbopogon caesius* STAPF, *Dicanthium annulatum* STAPF, *Heteropogon contortus* BEAUV. and *Themeda tremula* all the rest of the specimens examined have their ephelidial fructification in their inflorescence. In *Oryza sativa*, however, ephelidial fructification is often found also on the upper surface of top leaves of the plant. The measurements and their mean length of conidia from all of the twenty one hosts are presented in the table I.

TABLE I.

Conidial measurements and their mean length of Ephelis on twenty one host species of grasses.

Host	Conidia	Mean length
1. *Brachiaria distachya*	14.2—24.2 × 1.2—1.5	18.8
2. *Chrysopogon aciculatus* TRIN.	15.7—21.4 × 1.1—1.2	18.7
3. *Cymbopogon* sp.	17.1—30.0 × 1.0—1.4	24.1
4. *C. caesius*	18.5—27.1 × 1.0—1.5	23.4
5. *C. martnii* WATSON	14.2—21.4 × 1.1—1.2	18.1
6. *Dactyloctenium aegyptium*	15.2—28.5 × 1.1—1.3	17.2
7. *Dicanthium annulatum*	15.7—25.7 × 1.0—1.5	21.7
8. *Echinochloa crus-galli*	15.0—26.4 × 1.0—1.5	18.5
9. *Eragrostis tenuifolia*	11.4—21.4 × 1.2—1.3	16.2
10. *Hemarthria compressa* R. BR.	12.4—20.5 × 1.0—1.3	16.6
11. *Heteropogon contortus*	18.5—30.0 × 1.0—1.4	22.0
12. *Oryza sativa*	18.0—29.9 × 1.0—1.5	20.0
13. *Panicum repens* LINN.	16.2—24.2 × 1.0—1.2	20.1
14. *Paspalidium* sp.	14.2—20.0 × 1.0—1.1	16.0
15. *P. geminatum* STAPF	15.1—21.1 × 1.1—1.3	18.1
16. *Paspalum scrobiculatum* LINN.	14.2—21.4 × 1.1—1.3	18.0
17. *Pennisetum hohenackeri*	14.3—21.4 × 1.0—1.2	18.8
18. *Setaria pallide-fusca* STAPF & HUBB.	13.3—20.5 × 1.2—1.4	18.1
19. *Sorghum halepense*	12.8—22.8 × 1.0—1.2	18.5
20. *Sporobolus diander* BEAUV.	13.9—20.9 × 1.3—1.4	17.3
21. *Themeda tremula* HACK.	18.5—30.0 × 1.0—1.4	23.4

SPECIMENS EXAMINED

1. On leaves of *Brachiaria distachya*, very common in Bangalore, Mysore; September 10, 1945; leg. M. J. THIRUMALACHAR; and August 12, 1949; leg. H. C. GOVINDU.
   The epheliod state occurs as a white powdery striae on the upper surface of the top leaves. These leaves are often narrow and curved inwards. The inflorescence is rarely developed in such

infected plants and when they do, they remain mostly sterile.

2. On the inflorescence of *Chrysopogon aciculatus* (*Andropogon aciculatus*) Hazaribagh, Patna, Bihar; August 28, 1952; leg. M. J. THIRUMALACHAR. Fig. 5. This is the conidial stage of *Balansia andropogonis*, which is produced on the same culms later in the season.
3. On leaves of *Cymbopogon* sp., Torna Fort, Poona, Bombay; September 16, 1951; leg. M. M. PAYAK.
4. On leaves of *Cymbopogon caesium*, Hebbal, Bangalore, Mysore; August 30, 1950; leg. H. C. GOVINDU. Fig. 2.
   On this host, especially, a peculiar malformation results in the general appearance of the affected shoots of the host. This is commonly referred to as the ,,broomed" appearance and has been reported by DIEHL (1950) and PATEL et. al. (1951) on *Panicum clandestinum* and *Aristida* sp., respectively. The affected plants in the present instance, except for the peculiar type of dwarfing and phyllody are indistinguishable from the healthy plants. Further, the affected plants are characterised by shortened internodes resulting in dwarfing of the culms and by narrowed and smaller leaves giving the culms a ,,broomed" appearance.

This brooming appearance seems to affect the entire plant, or rarely a normal shoot may arise from a diseased plant. The ephelidial fructification is usually present in the inner surface of the sheathing leaves. However, it is often seen that plants which have recovered from infection of *Balansia* develop the "broomed" appearance (Fig. 2). In such instances, it is possible that this is of great significance in dissemination of the fungus, since *Ephelis* conidia are produced in larger numbers than ascospores of *Balansia* stage. Further, the latter stage seldom develops during the early summer, while the *Ephelis* stage develops both in summer and during the rainy season.

There is no definite development of typical exciple, but the conidia arise from the conidiophores, which are developed from the stromatic layer of delicate hyphae, lining the inner surface of the sheathing leaves of malformed structure.

5. On inflorescence of *Cymbopogon martnii*, Nandi Hills, Mysore; October 28, 1953; leg. H. C. GOVINDU.
6. On inflorescence of *Dactyloctenium aegyptium*, Patna, Bihar; November 14, 1952, leg. M. J. THIRUMALACHAR; Bangalore, Mysore; October 25, 1953; leg. H. C. GOVINDU. Fig. 10.
7. On leaves of *Dicanthium annulatum*, Netrahat, Bihar; December 6, 1952; leg. M. J. THIRUMALACHAR. The infected plants do not, ordinarily develop inflorescence, but are reduced to leafy shoots, presenting symptoms of *Ephelis* on *Cymbopogon caesium*.
8. On inflorescence of *Echinochloa crus-galli*, Patna, Bihar; November 14, 1952; leg. M. J. THIRUMALACHAR. Fig. 12.

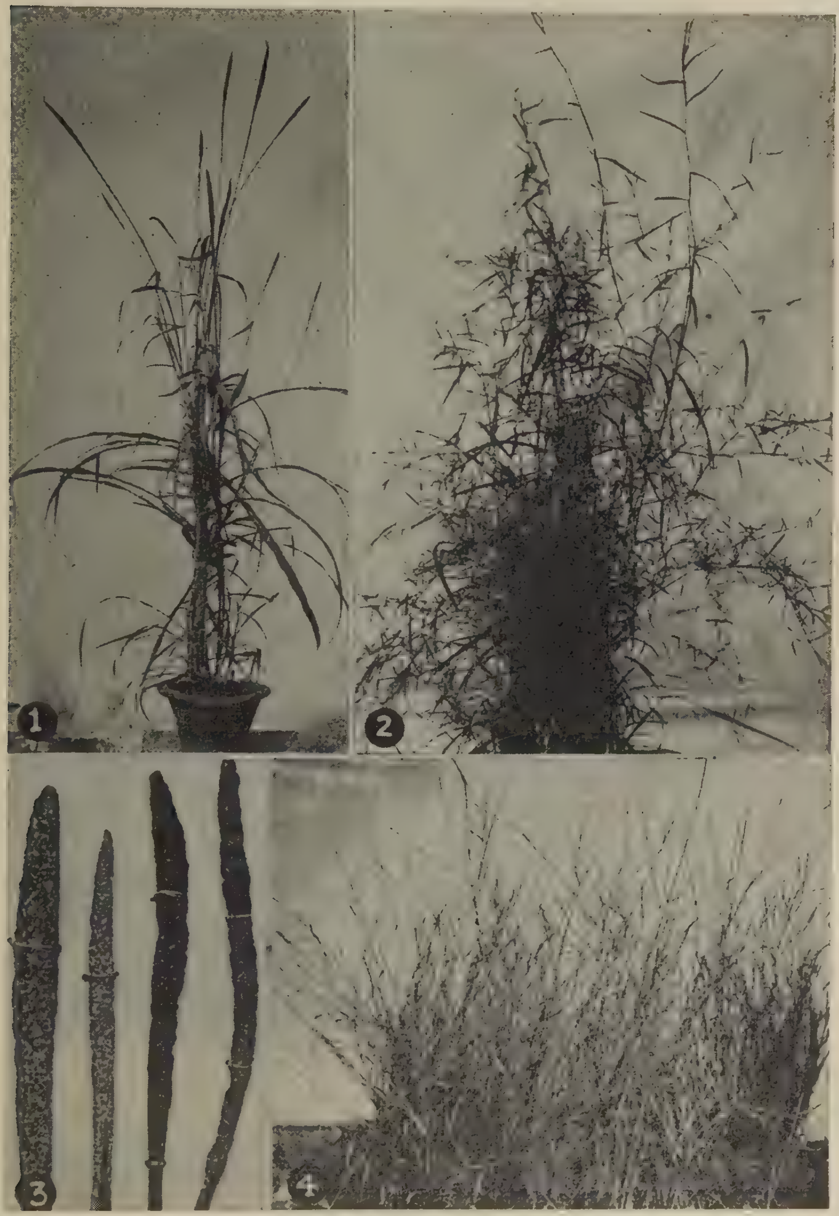
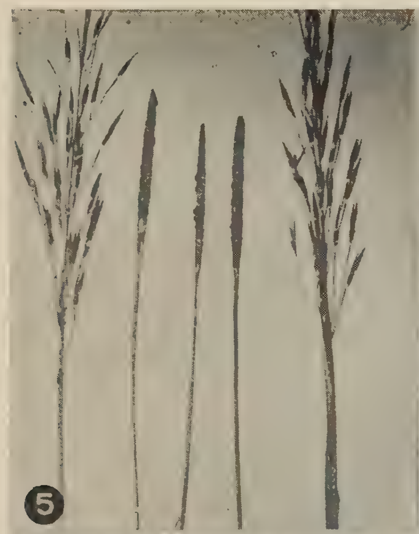
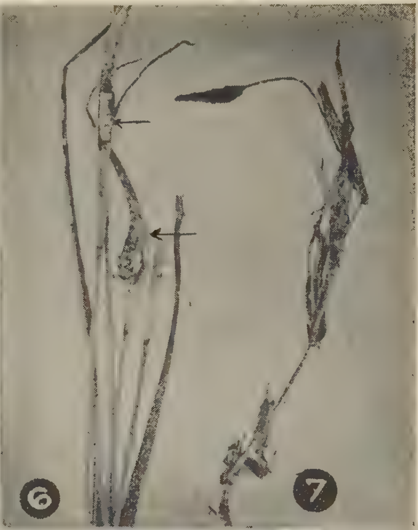
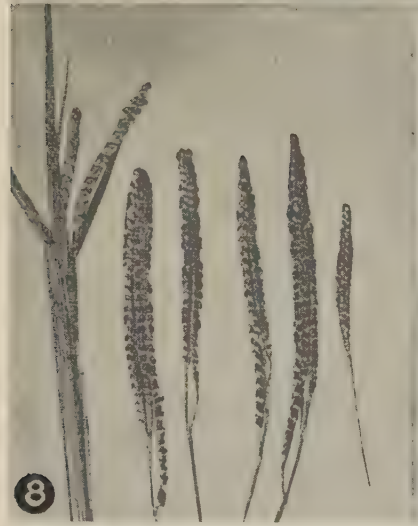


PLATE I.

Fig. 1. Infected plant of *Sorghum halepense* with ephelidial fructification at apex of culms surrounding aborted inflorescence.
Fig. 2. 'Broomed' appearance of *Cymbopogon caesium* affected with *Ephelis*.
Fig. 3. Malformed panicle of *Sorghum halepense* bearing ephelidial fructifications.
Fig. 4. Healthy and diseased culms of *Eragrostis tenuifolia*.







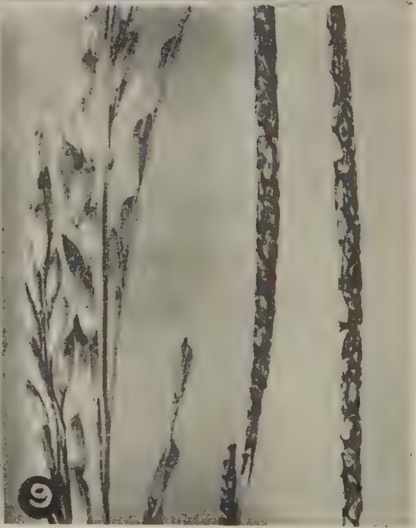


PLATE II.

Fig. 5. Healthy and diseased inflorescence of *Chrysopogon aciculatus*.

Fig. 6. A portion of healthy inflorescence of *Hemarthria compressa* on the left and aborted inflorescence developed as a result of *Ephelis* infection on the right.

Fig. 7. *Ephelis* on *Setaria pallide-fusca*.

Fig. 8. Healthy inflorescence of *Paspalum scrobiculatum* on extreme left, the rest are all infected with *Ephelis*.

Fig. 9. Healthy and diseased inflorescence of *Oryza sativa*.

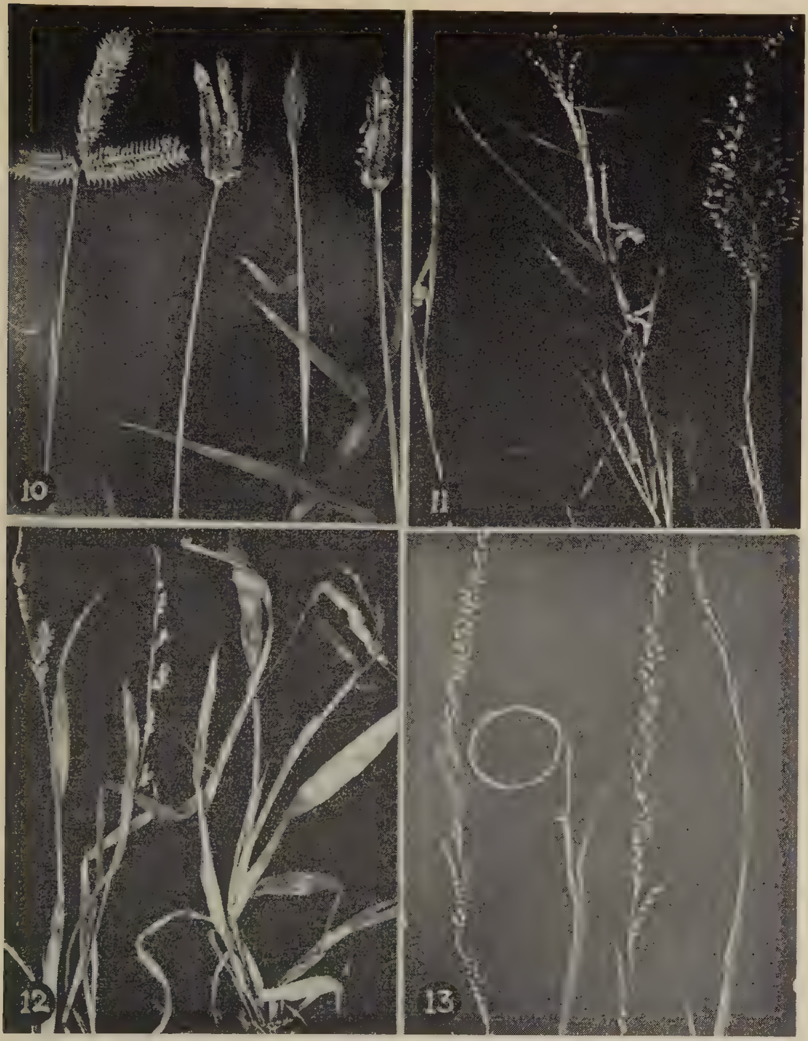


Plate III.

Fig. 10. Healthy and diseased inflorescence of *Dactyloctenium aegyptium*.
Fig. 11. Inflorescence of *Sporobolus diander* covered by ephelidial fructification causing dwarfed and sterile spikelets (at left and middle) and comparable with normal spikelets (at right).
Fig. 12. *Ephelis* on *Echinochloa crus-galli*; healthy inflorescence on left and diseased on right.
Fig. 13. *Ephelis* on *Paspalidium geminatum*.

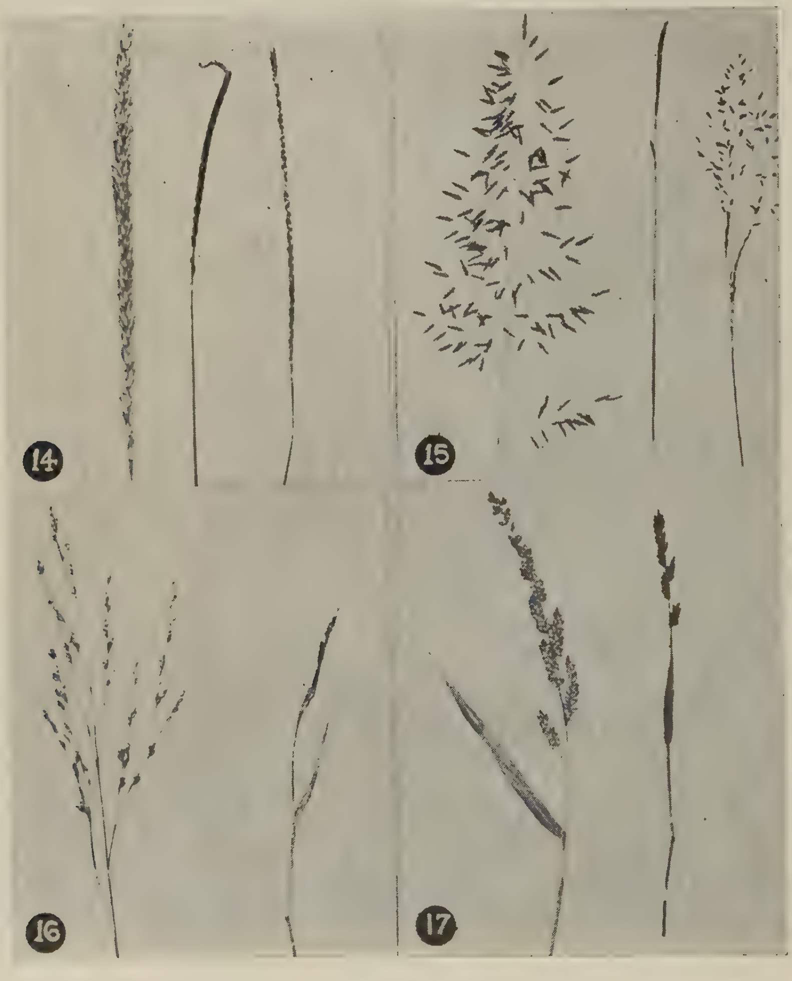


Plate IV.

Fig. 14. Healthy and diseased inflorescence of *Pennisetum hohenackeri*.
Fig. 15. Healthy inflorescence of *Eragrostis tenuifolia* on left and ephelidial fructification on the entire inflorescence (at middle) and individual spikelets on the right.
Fig. 16. Healthy and diseased inflorescence of *Panicum repens*.
Fig. 17. Healthy and diseased inflorescence of *Paspalidium* sp.

9. On inflorescence of *Eragrostis tenuifolia*, Hebbal, Bangalore, Mysore; October 28, 1953; leg. H. C. GOVINDU. Figs. 4 and 15.
10. On inflorescence of *Hemarthria compressa*, Yeshavantapur, Bangalore; December 30, 1946; leg. M. J. THIRUMALACHAR. Fig. 6.
11. On leaves of *Heteropogon contortus*, Parasnath, Bihar; December 18, 1952; leg. H. C. GOVINDU.
12. On mostly inflorescence and rarely on upper surface of top leaves of *Oryza sativa*, Hebbal, Bangalore; January 28, 1953; leg. H. C. GOVINDU. Fig. 9.
13. On inflorescence of *Panicum repens*, Patna, Bihar; November 18, 1952; leg. M. J. THIRUMALACHAR. Fig. 16.
14. On inflorescence of *Paspalidium* sp., Patna, Bihar; November 1, 1952; leg. M. J. THIRUMALACHAR. Fig. 17.
15. On inflorescence of *Paspalidium geminatum*, Hebbal, Uttarahalli, Bangalore, Mysore; October 30, 1949; leg. H. C.GOVINDU and M. J. THIRUMALACHAR. Fig. 13.
16. On inflorescence of *Paspalum scrobiculatum*, Hebbal, Bangalore; October 28, 1953; leg. H. C. GOVINDU. Fig. 8. The racemes of inflorescence in the diseased culm are usually more or less glued together.
17. On inflorescence of *Pennisetum hohenackeri*, Yadiur and Hebbal, Bangalore, Mysore; October 30, 1952; leg. H. C. GOVINDU. Fig. 14.
18. On inflorescence of *Setaria pallide-fusca*, Patna, Bihar; October 14, 1952; leg. M. J. THIRUMALACHAR. Fig. 7.
19. On inflorescence of *Sorghum halepense*, Hessarghatta, Mysore; October 20, 1953; leg. H. C. GOVINDU. Figs. 1 and 3.
20. On inflorescence of *Sporobolus diander*, Patna, Bihar; October 14, 1952; leg; M. J. THIRUMALACHAR. Fig. 11.
21. On upper surface of top leaves of *Themeda tremula*, near Dupadagiri shola, Kemmangundi, Mysore; May 5, 1954; leg. H. C. GOVINDU.

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THE COMPARATIVE MORPHOLOGY OF THE FUNGAL MAT AND BALL OF TRICHOPHYTON RUBRUM

by

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(with 3 figs.)

(16.XII.1960)

Trichophyton rubrum, (CDC strain), seeded onto an agar surface, grows in the form of a slightly convex mat. When the fungus is seeded into a liquid medium and agitated on a rotary shaker, spheroidal clones appear. Freeze-dried specimens of both forms were sectioned, studied and photographed. To our knowledge this constitutes the first such study of a dermatophyte; the findings are described in this report.

Materials and Methods

Fifteen ml of Nickerson's growth medium (Nickerson & Chadwick (1946)) containing 1.5 % agar were dispensed into a sterile Petri dish. After hardening, the medium was centrally inoculated with a loopful of fungal homogenate (Vicher, Lyon & White (1959)) prepared from a 7 day old culture of *T. rubrum* and incubated at 30° C until the colony size was at least 3 cm (attained in approximately 10 days). This same medium, *sans* agar, as well as a chemically defined medium (Zussman (1959)), was used for the liquid cultures; 135 ml of these media were dispensed into separate sterile 250 ml Erlenmeyer flasks. Each flask was inoculated with 0.1 ml of the fungal homogenate and incubated at room temperature for at least three weeks, with continuous shaking, until well-defined, pigmented clones were observed.

A strip 5 mm wide, cut from the center of the mat, was instantly frozen in an isopentane bath immersed in liquid nitrogen. Whole spheroids were treated similarly. The frozen materials were dessicated *in vacuo* for several days and imbedded in paraffin. Sections, 10 μ thick, were mounted on slides and cleared in xylol. Previous studies (Zussman, Lyon & Vicher (1960)) showed that this solvent

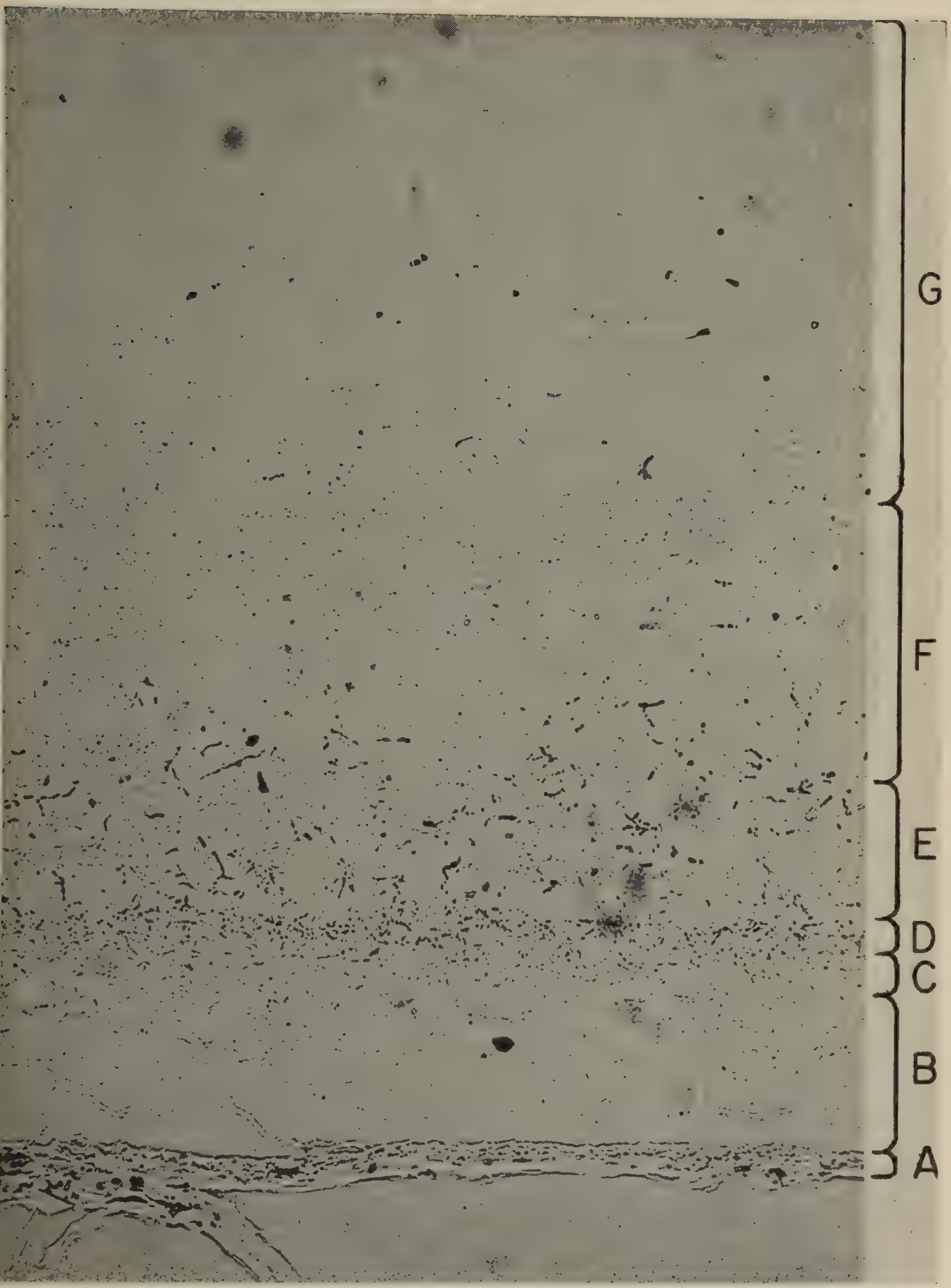

Fig. 1. Cross-section of a pigmented, three week old fungal mat. 330 ×.

does not dissolve the pigments of *T. rubrum.* Photomicrographs were made on Kodak M plates with a green filter for contrast.

RESULTS

Figure 1 is a cross-section of a mature fungal mat. Seven distinct zones may be seen:

(A) paper used in the preparation of the freeze-dried specimen,

(B) submerged, vegetative, non-pigmented mycelia with a few extracellular bodies containing a purple pigment,

(C) a relatively non-compact arrangement of hyphae, a few of which have yellow walls,

(D) many closely-packed hyphae with yellow walls and great numbers of intramural and extracellular melanin-like granules; the hyphae which bear the dark pigment granulae are often enlarged and grossly distorted, resembling "bags full of marbles" (fig. 2),

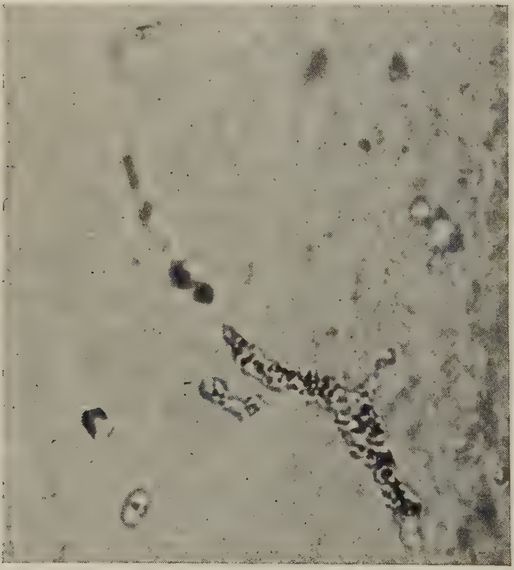

Fig. 2. Cross-section of a pigmented fungal mat showing characteristic enlarged granule-filled hypha. 1200 ×.

(E) a transition zone in which the semi-compact hyphae contain fewer pigment granules and yellow filaments, with a very few extracellular granules and not many atypical hyphae,

(F) the great mass of the fungal colony, consisting of non-pigmented, non-reproductive hyphae, and

(G) the uppermost layer composed of hyphae that bear the reproductive structures, chlamydospores and microconidia. Macro-

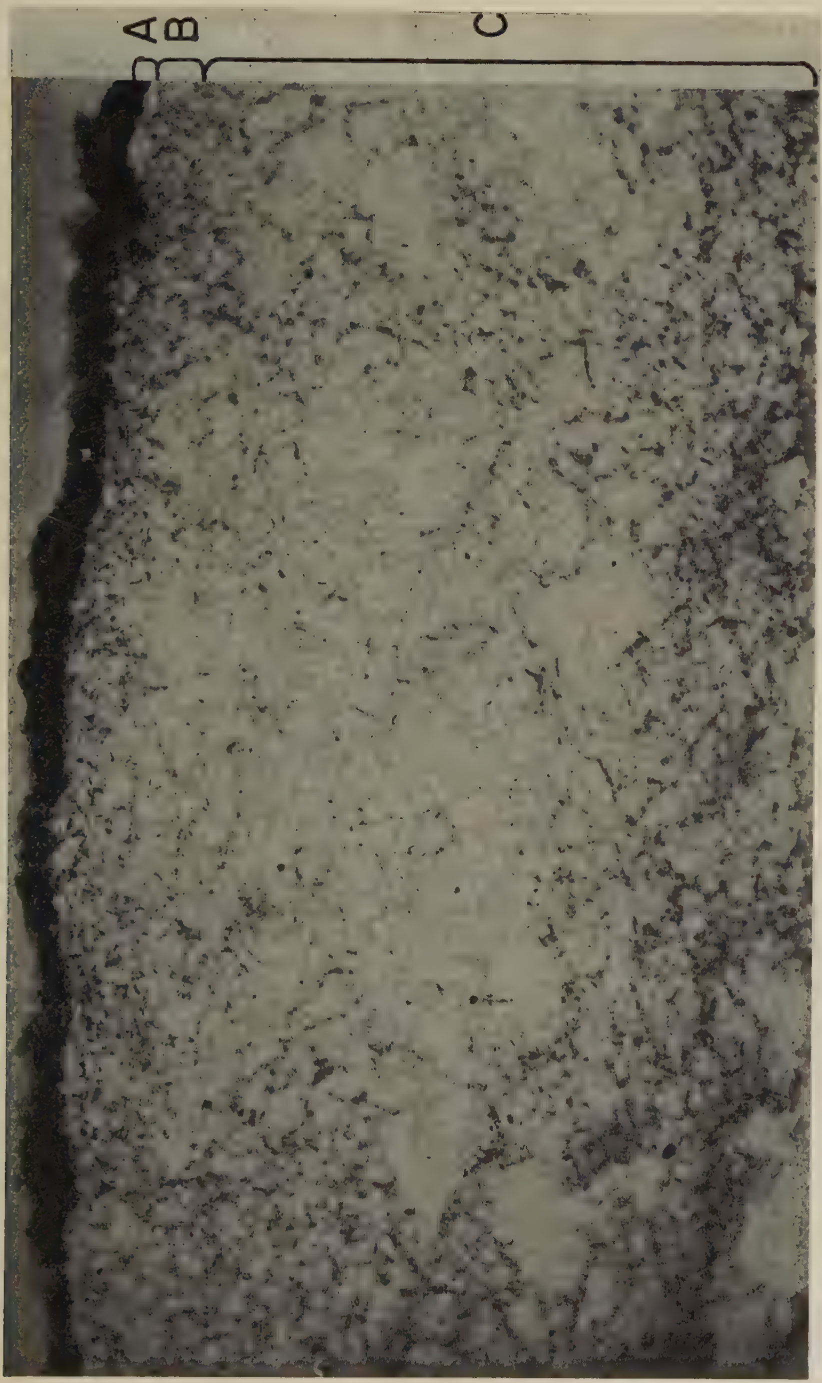

Fig. 3. Cross-section of a fungal spheroid. 330 ×.

conidia have not been demonstrated in the CDC strain of *T. rubrum* used in this study.

The gross morphology of the fungal spheroid is different from that of the mat, but similarities exist on the cellular level. The spheroid (figure 3) is composed of three zones:

(A) an outermost layer which is thin, compact, physically tough and highly pigmented; no pigment granules are found free in the extracellular spaces,

(B) a transitional zone in which about 15 % of the hyphal walls are yellow, and

(C) a watery, non-compact, non-pigmented zone comprising most of the fungal volume. No reproductive structures of any kind have been observed in this, or any other zone of the spheroidal form.

Discussion

Comparison of figures 1 and 3 reveal both similarities and dissimilarities between the fungal mat and spheroid. There can be little doubt that a morphological relationship exists between structures of zones (D) of the mat and (A) of the spheroidal form, and of (E) and (B), and (F) and (C) of the mat and spheroid, respectively.

From these observations, one may conclude that the spheroid is equivalent to an involuted fungal mat, folded in such a way that the internal portion of the spheroid is analogous to the aerial mycelia of the mat, and the outer zone of the former to the undersurface of the mat. These structural similarities suggest similarities in function.

The manner in which the mat and spheroidal forms develop is not the same. Several days after the inoculation of a Petri dish, vegetative mycelia may be seen in the medium, followed by tufts of white aerial mycelia directly over the point of inoculation; no differentiation into other specialized components is seen at this time.

After several days, the diameter of the colony increases and the central regions appear to be far more dense than the periphery. Shortly thereafter, yellow hyphae appear centrally and extend concentrically with the passage of time. Later, grotesque, enlarged, granule-bearing hyphae may be seen in the center of the clone. Finally, at morphological maturity the spread of the granule-bearing zone overtakes new peripheral growth as well as the peripheral boundaries of the yellow hyphae. This results in the fusion of these two zones into a single one which now extends almost to the periphery of the clone. In summary, growth, differentiation and maturation proceed from the point of inoculation (the oldest area) to the youngest areas which are the last to differentiate and mature.

In the initial growth period of the spheroidal clone, the mycelia extend from the nidus to form a stellar clone that is centrally dense. When the clone reaches optimal size [1]), peripheral growth ceases and is followed by internal growth tending to increase the cellular density of the clone. An early result of this process is the marked, localized thickening of the periphery which causes the clone to lose its early characteristically stellar appearance. Little or no increase in clonal size occurs thereafter. Grossly, hard-walled spheroids with fluid-saturated centers are seen and intramural pigmentation begins, terminating in an appearance similar to that seen in figure 3. Further incubation, with or without shaking, is characterized by the release of one or more conical fragments from a majority of the spheroids. The culture becomes a mixture of conical fragments, punctured spheroids and bubbles of gas. The apex of the conical fragment corresponds to the center of the clone, and the base to its periphery. The significance of these morphological forms and the origin of the gas bubbles are not known. The latter may merely represent air entrapped during shaking or may be a product of fungal metabolism. That this organism has a marked capacity for reductive metabolism is indicated by the formation of arsine gas in a medium containing arsenate (ZUSSMAN, VICHER & LYON (1961).

Other studies (ZUSSMAN (1959); ZUSSMAN, LYON & VICHER (1960)) have shown that several different pigments were produced by the two morphological forms of *T. rubrum*. Reconciliation of morphological differences with the various classes of pigment produced (e. g., anthraquinoid, melanoid, and perhaps others) remains a problem of considerable magnitude and interest.

SUMMARY

A CDC strain of *Trichophyton rubrum* was grown on solid medium and in shake cultures. The resulting forms (mat-like and spheroidal, respectively) were prepared for sectioning and study by the freeze-drying technic. The results indicate certain similarities and differences in growth and pigmentation patterns, depending upon the method of culture. Pigmentation is not random but occurs in specific areas of the fungal clone. The relationship between each form and their pigmentation patterns is briefly described and discussed.

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[1]) Optimal size depends upon the number of clones in the medium, flask size, composition of the medium, flask shaking rate, temperature and other factors.

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NUTRITIONAL AND ENVIRONMENTAL FACTORS AFFECTING PIGMENTATION IN *TRICHOPHYTON RUBRUM*

by

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(with 1 fig.)

It has been suggested that pigmentation of the dermatophytes is dependent upon nutritional and environmental factors. For example, the specific requirements for carbohydrates in pigmentation and in growth have been reported by Lewis & Hopper (1941); Vicher, Lyon & White (1959) and Zussman, Lyon & Vicher (1960). In addition, the effect of certain amino acids on pigmentation has been described by Silva (1953, 1958), by Zussman, Lyon & Vicher (1960), and others. Thus it appears that pigmentogenic patterns are not random occurrences, but are due to the influences of growth medium, availability of atmospheric oxygen, and probably other factors.

The effects on pigmentation of amino acids, carbohydrates, vitamins, oxygen and light are described in this communication.

MATERIAL AND METHODS

A homogenate of *Trichophyton rubrum* (CDC strain), previously described by Vicher, Lyon & White (1959) was prepared from a 6 day old culture.

The media employed in this study are described in table I and in the text below. The media bases were sterilized by autoclaving at 121° C for 20 minutes. Carbohydrates, when required, were autoclaved as separate aqueous solutions and added aseptically; vitamins were sterilized by Seitz filtration.

The amino acids were tested for their effects on pigmentation in Medium I. This medium contains ammonium nitrate (1.5 g/l) as the nitrogen source, and glucose (40 g/l) as the source of carbon. The amino acids (table II) were added separately to the above medium in experiments 1 and 2, and 10 ml samples of each medium

TABLE I.

Composition of Media

Medium	Composition or Reference
I	GEORG & CAMP (1957)
IA	I with a single amino acid in an amount equal to its concentration found in natural trypose *)
IB	IA with amino acid concentration equivalent to nitrogen contained in 0.52 g/l of tyrosine
II	NICKERSON & CHADWICK (1946)
IIA	II modified by omission of 5 % glucose
IIB	IIA with 0.5 % L-sorbose in place of D-glucose
III	ZUSSMAN (1959), contains amino acids and vitamins listed in table II, experiment 1, and table III
IIIA	III without vitamins
IV	Sabouraud's dextrose agar

*) The analysis for Bacto-Tryptose was supplied by Difco Laboratories, Inc., Detroit, Michigan.

were dispensed into Petri dishes and allowed to harden. One loopful of the fungal homogenate was placed at the center of the dish and the cultures were incubated at 30° C for 18 days.

Five media were used to determine the carbohydrate requirement for pigmentation: I with 0.52 g/l of tyrosine, II, IIB, III, and IV.

The effect of glucose on the growth rate of *T. rubrum* was observed in Medium II containing asparagine (1 g/l; designated A), tryptose (10 g/l; T), yeast extract (0.01 g/l; Y), and glucose (50 g/l; G) in the combinations ATY, GTY, GAT, GAY, and GATY; and in Medium III (GAT*Y* where T* and Y* indicate synthetic tryptose and a vitamin substitute for yeast extract respectively [1]).

The effects on pigmentation of biotin, nicotinic acid, pyridoxine, riboflavin, and thiamin were studied in Medium I with added tyrosine and in Medium IIIA supplemented with the vitamins added singly and in combination.

The effect of ascorbic acid on pigment formation was studied by incorporating various concentrations of this reducing agent (from 0.001 to 5 g/l) into Medium I with tyrosine. The hardened medium was centrally inoculated and periodically examined for growth and pigmentation.

The effect of atmospheric oxygen on pigmentation was studied in cultures grown on Medium I with tyrosine, and on Medium II, III, and IV. Ten ml portions of the above media were hardened in

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[1] The vitamin substitute was prepared to contain the percentage composition of these substances in yeast extract obtained from Difco Laboratories, Inc., Detroit, Michigan.

Petri dishes and inoculated centrally with a loopful of homogenate. The cultures were then immediately placed into a vacuum dessicator jar which was alternately flushed and filled 6 times with illuminating gas and then flushed with pure nitrogen 4 times. On the following day the jar was again flushed and filled with nitrogen and incubated at room temperature. In another experiment, cultures were grown aerobically until colonies approximately 20 mm in diameter grew out, and were then placed into a nitrogen atmosphere as described above. Other cultures were alternately grown under anaerobic conditions for several consecutive periods.

The pigmentation of *T. rubrum* in liquid culture (ZUSSMAN, VICHER & LYON (1961)) was studied in Medium I with tyrosine and in Medium II and III. The cultures contained 135 ml of medium in a 250 ml Erlenmeyer flask inoculated with 0.2 ml of fungal homogenate, and they were incubated at 26° C. One-half of the cultures were incubated for 2 months without shaking while the others were agitated on a rotary shaker at about 150 rpm for a similar period of time. Other flasks were initially incubated without shaking for two to three weeks and then were shaken for the remainder of the incubation period.

The effect of light on pigmentation was observed qualitatively. Petri dishes containing Medium II were inoculated with fungal homogenate; half were wrapped in a double layer of aluminum foil and the others were left unwrapped. All the cultures were placed, bottom up, in direct sunlight several hours per day. The temperature varied between 26° C and 28° C depending upon the ambient room temperature. The cultures were examined weekly for growth and pigmentation. In another experiment, 40 ml of Medium II were dispensed into 160 mm Petri dishes and 2 ml of fungal homogenate were spread over the entire surface of the hardened agar. One-half of the cultures were placed into a large, light-proof container while the remaining cultures were partially sealed with adhesive tape to retard loss of moisture but remained exposed to ordinary light. All the cultures were incubated at room temperature for 6 weeks when the degree of pigmentation was evaluated.

RESULTS AND DISCUSSION

Amino Acid Studies: The effect of various amino acid supplements in Medium I (in which pigmentation does not ordinarily occur) is reported in table II. After 6 days of incubation, pigmentation was observed in the media with arginine, phenylalanine, and tyrosine. (experiment 1). The degree of pigmentation seen with tyrosine approached that observed in a 6 day culture in a complete medium. It is interesting to note that inhibition of growth occurred with cysteine or methionine ²). After an additional 12 days fungal pig-

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²) The inhibition of growth in the cysteine-containing medium was absolute. However, the methionine culture revealed a sparse, fern-like growth under microscopic examination. Inhibition of growth by methionine occurs only in minimal media, for it was shown that concentrations four times in excess of that which inhibits in Medium I failed to inhibit growth when added to Medium II, a complete medium.

ment was also noted in Medium I containing aspartic acid, leucine, or valine. It is clear that maximal pigmentation occurred with tyrosine; with phenylalanine a lesser degree of pigmentation was noted.

In experiment 2, after 6 days of incubation, pigmentation was noted in cultures containing tryptophane. Inhibition of growth was again observed in the presence of cysteine or methionine.

Table II.

Production of Pigment by T. rubrum on a Basal Medium Containing Amino Acid Supplements

Amino Acid Supplement	Concentration (g/l)	Pigmentation	
		6 Days	18 days
		Experiment 1	
Medium I		—	—
Arginine	0.50	(Y) +	(Y, R) +
Aspartic Acid	0.68	—	(Y) +
Cysteine	0.20	Growth Inhibited	
Glutamic acid	1.54	—	(Y) ±
Glycine	0.70	—	—
Histidine	0.18	(Y) ±	(Y) ±
Isoleucine	0.40	—	—
Leucine	0.74	—	(Y) +
Lysine	0.60	—	—
Methionine	0.22	Growth Inhibited	
Phenylalanine	0.36	(Y) +	(Y) ++
Proline	0.10	(Y) ±	(Y) ±
Serine	0.10	(Y) ±	(Y) ±
Threonine	0.32	—	(R) +
Tryptophane	0.10	—	—
Tyrosine	0.52	(Y) +++	(Y, R) ++++
Valine	0.52	—	(Y, R) +
		Experiment 2	
Arginine	0.12	—	—
Aspartic acid	0.38	—	—
Cysteine	0.35	Growth Inhibited	
Glutamic acid	0.42	—	(Y) ±
Glycine	0.21	—	—
Histidine	0.14	—	—
Isoleucine	0.37	—	—
Leucine	0.38	—	(Y) ±
Lysine	0.21	—	—
Methionine	0.43	Growth Inhibited	
Phenylalanine	0.48	(Y) ++	(Y, R) ++++
Proline	0.33	—	—
Serine	0.33	—	—
Threonine	0.34	—	—
Tryptophane	0.29	—	(Y) +
Tryosine	0.52	(Y) ++++	(Y, R) ++++
Valine	0.33	—	—

Y, yellow pigment; R, red; ++++, maximum pigmentation observed; +, minimum pigmentation observed; ±, pigment production questionable; — no pigmentation observed.

These findings suggest that tyrosine and phenylalanine may serve as precursors and/or intermediates in the metabolic pathways resulting in pigment formation of *T. rubrum*. The fact that tyrosine supports more rapid and intense pigmentation than phenylalanine indicates the former amino acid may be metabolically closer to the specific pigment precursor(s) than the latter. In experiment 1, several amino acids supported pigmentation, while in experiment 2, only the aromatic amino acids functioned in this way. The amino acid concentrations were higher in every instance where pigment was produced. This phenomenon may indicate that amino acids, not ordinarily utilized as intermediates for pigment formation, when incorporated into a medium in concentrations greater than necessary to support growth, are converted by *T. rubrum* into aromatic amino acids, or other pigment intermediates. Furthermore, the higher concentrations of the non-aromatic amino acids may tend to "spare" the aromatic ones, thereby increasing the effective concentration of the latter for pigmentation.

In several instances contaminating microorganisms grew adjacent to a fungal colony on Medium I. It was then observed that pigmentation often occurred in that portion of the *T. rubrum* colony next to the contaminant. These organisms were either gram positive bacilli or yeasts. Furthermore, the intensity of pigmentation was often inversely proportional to the distance of the contaminant from the fungus. It appears that cross-feeding may occur involving certain diffusible substances produced by the contaminating organism and utilized by the fungus for pigment production. Apparently the dermatophyte is unable to synthesize these metabolic intermediates when growing on minimal media. The diffusible intermediate(s) may be amino acids and/or their precursors.

Carbohydrate Studies: A variety of monosaccharides appear capable of supporting pigment production (LEWIS & HOPPER (1941) and ZUSSMAN (1959)). In our studies the exclusion of **D**-glucose or **L**-sorbose from any of the media used resulted in a complete lack of pigment formation. However, the pigment formed with **L**-sorbose was dark brown instead of the characteristic red. It should be noted that although **L**-sorbose supports pigment production as well as glucose, at concentrations greater than 0.5%, the former sugar caused an inhibition in the growth of *T. rubrum* which could not be reversed by increased concentrations of glucose. These observations suggest that carbohydrate may be required primarily as a source of energy in pigmentation rather than a source of carbon atoms for pigment molecules.

Growth Rate Studies: A generally low rate of growth was observed in carbohydrate-free media (figure 1), confirming the observations of VICHER, LYON & WHITE (1959). Following incubation for 10-12 days in the Medium II combinations GATY, GAT*Y*, GTY, and GAT, the fungal masses are approximately equivalent (over 400 mg). Conversely, ATY (glucose-free) supported

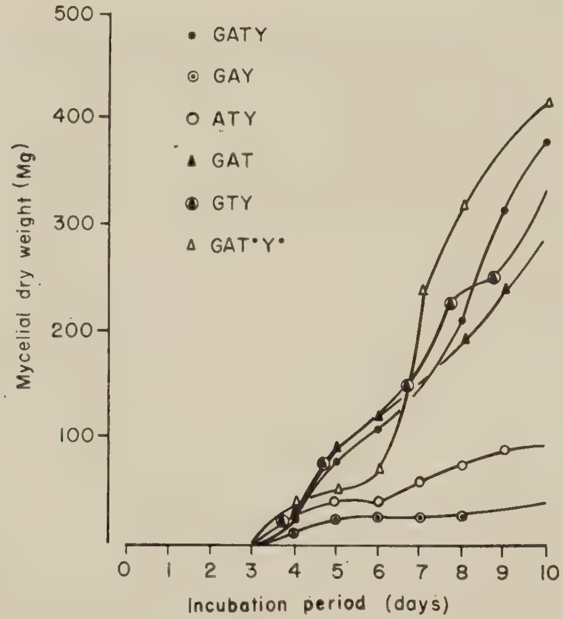

Fig. 1. Growth Curves of *Trichophyton rubrum* in Various Media (see text for additional information)

less than 100 mg of growth under otherwise similar conditions, and no pigment was observed. Under the conditions of this experiment, asparagine is a poorly utilized source of nitrogen for *T. rubrum.* The nitrogen contributed by the yeast extract, 0.01 g/l, is negligible. The curves in figure 1 indicate that a medium containing only glucose and tryptose provides the basic requirements for the growth of *T. rubrum.*

Vitamin Studies: The observation that biotin, nicotinic acid, pyridoxine, riboflavin and thiamin present over a wide concentration range (table III), have no apparent effects on pigment

Table III.

Concentrations of Vitamins

Vitamin	Maximum Concentration		Minimum Concentration
		(Micrograms per liter)	
Biotin	40		4
Nicotinic Acid	710		71
Pyridoxine	280		28
Riboflavin	560		56
Thiamin	160		16

production supports the work of Silva (1953). This finding strongly suggests that *T. rubrum* is fully capable of synthesizing its entire complement of required coenzymes.

Ascorbic acid has been shown to inhibit melanin formation in goldfish (Ito (1952)). This reducing agent seems to have a two-fold inhibitory effect on *T. rubrum*: it may inhibit growth and/or pigmentation (Table IV)[3]. At concentrations between 0.01 and 0.25 g/l some growth occurs but pigmentation is delayed and less intense than observed in control cultures. Between 0.0025 and 0.01 g/l growth is uninhibited while pigmentation is again somewhat less than normally seen. Concentrations lower than 0.0025 g/l do not inhibit growth or pigment formation. It has been observed that ascorbic acid does not act directly upon the pigment(s) to reduce them to leuco compounds.

Table IV.

Inhibitory Effects of Ascorbic Acid on Growth and Pigmentation of T. rubrum

oncentration of Ascorbic Acid (g/l)	Degree of Growth			Degree of Pigmentation		
	6 Days	9 Days	13 Days	6 Days	9 Days	13 Days
	—	—	—	—	—	—
	—	—	—	—	—	—
	—	—	—	—	—	—
	—	—	—	—	—	—
	+	+	+	—	—	1/4 (Y)
5	+	+	+	1/3 (Y)	1/3 (Y)	1/2 (Y)
00	++	++	++	1/2 (Y)	1/2 (Y)	1/2 (Y)
25	++	++	+++	(Y)	(Y)	(Y)
2	+++	+++	+++	(Y)	(Y)	(Y)
56	+++	+++	++++	(Y)	(Y)	(Y)
00	++++	++++	++++	(Y)	(Y)	(Y)
25	++++	++++	++++	(Y)	(Y)	(Y, R)
0	++++	++++	++++	(Y, R)	(Y, R)	(Y, R)
00 (control)	++++	++++	++++	(Y, R)	(Y, R)	(Y, R)

symbols have the following meanings: —, lack of growth or pigmentation; +, minimal growth; +, maximal growth; 1/4, 1/3, or 1/2, the approximate fraction of cultures exhibiting pigment; , yellow or red pigment respectively.

Aerobiosis-Anaerobiosis Studies - Effect on Growth: Atmospheric oxygen is necessary for the initial growth of *T. rubrum* since no growth was observed in any of the cultures incubated anaerobically. Young cultures, first grown aerobically, were able to continue to grow at a diminished rate after being placed into an anaerobic environment (table V).

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[3] Due to the thermolability of ascorbic acid and the ease with which it is oxidized reproducible results can be obtained only if care is taken to (a) prevent oxidation of the acid prior to its incorporation into the medium, and (b) add the ascorbic acid into well-cooled media.

Table V.

Growth of T. rubrum under anaerobic conditions

Experiment No.	Number of Cultures	Treatment	Average Colony Diam. mm		
			Initial	10 days	20 days
1	6	a	—	265	600
2	4	a	—	360	780
3	12	b	—	0	0
4	10	c	200	260	320
5	8	c	330	500	—
6	5	c	165	190	280
7	6	c	360	500	590
8	4	c	300	350	480
9	4	c	375	460	500
10	4	c	420	440	520

a: aerobic controls; b: incubated anaerobically throughout the experimental period; c: first grown aerobically until an arbitrary initial size was attained, then placed in an anaerobic atmosphere for the remainder of the experiment.

Effect on Pigmentation: Pigmentation, in *T. rubrum*, is an aerobic process, since it never occurs under anaerobic or microaerophilic conditions. Anaerobiosis does not irreversibly denature the pigment-forming mechanism; the studies reported here indicate that some of the steps leading toward pigmentation may proceed during either aerobic or anaerobic growth. Non-pigmented, young cultures, initially grown under anaerobic conditions become pigmented rapidly after a short period (48-96 hours) of aerobic incubation. Apparently certain precursors form and accumulate during anaerobic growth only requiring the subsequent introduction of oxygen to carry the pigmentation process to completion.

The oxygen requirement for pigment production became manifest in both solid and liquid cultures. Under alternate aerobic and anaerobic conditions, *T. rubrum*, in solid cultures, developed a series of concentric rings on the underside of the colony in which the pigmented rings were formed during periods of aerobiosis and the non-pigmented rings in periods of anaerobic incubation. In the older areas of a giant colony there appeared to be a loss in pigment-producing capacity.

In the liquid cultures no pigmentation was ever observed within the medium during stationary incubation. As reported by Silva (1953) and confirmed in these studies, fungal masses adhering to the sides of the flasks near the air medium interface, as well as those floating on the surface of the medium, became normally pigmented. When the flasks were agitated on a rotary shaker, the fungal spheroids (Zussman, Vicher & Lyon (1961)) were uniformly and highly pigmented after 20 days. Pigmentation did not occur in those media lacking glucose even after 2 months of incubation.

The observation that pigmentation is an aerobic process is supported by the finding that concentrations of ascorbic acid that do not suppress growth are able to partially suppress pigment formation (*vide supra*).

Comment: It is clear from the literature that trace metals are necessary for certain fungi (FOSTER (1949) and ENGLISH & BARNARD (1955)), and in certain pigment-forming systems (e.g., the copper requirement for tyrosinase activity, SARATA (1935)). No definitive studies relating to the trace metal requirements were carried out. However, in anticipation of a demonstrated requirement for trace metals, particularly since pigmentation in *T. rubrum* appears to involve the tyrosinase system (ZUSSMAN, LYON & VICHER (1960)), tap water was used in the preparation of the various media. The use of tap water resulted in more uniform pigmentation and growth patterns than did distilled water.

SUMMARY

1. Pigmentation in *Trichophyton rubrum* appears to be dependent upon tyrosine and phenylalanine (as precursors), carbohydrate (primarily as an energy source), and oxygen.

2. Cysteine and methionine inhibit the growth of *T. rubrum* in a minimal medium. **L**-sorbose also inhibits the growth of this organism, even in a complete medium.

3. *T. rubrum* apparently synthesizes all of the vitamins required for growth and pigmentation.

4. At certain concentrations of ascorbic acid, it is possible to distinguish between the pigmentogenic and growth processes of *T. rubrum*.

5. Sunlight suppresses growth and pigmentation in *T. rubrum*.

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A NEW AND WIDELY DISTRIBUTED SPECIES OF PSEUDOARACHNIOTUS ¹), ²).

by

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(with 36 figs.)

(23.XII.1960)

During the last several years the authors have been engaged in a study of the Gymnoascaceae. This study has usually been seriously hampered by a paucity of cultures. Often there was available for study only one or two strains of a given species, and, as a result, almost no concept of strain variation could be formulated. This difficulty now has been overcome in many instances occasioned by the isolation of a large number of Gymnoascaceae by one of us (GFO). It is our belief that the isolation techniques presented in this paper will enable investigators throughout the world to contribute to the fund of knowledge regarding the Gymnoascaceae, a family which heretofore has been quite neglected with respect to taxonomic, morphological, physiological and genetic studies.

MATERIALS AND METHODS

The isolation of Gymnoascaceae from soil was accomplished in the following manner. A 1 : 10 dilution of the soil was prepared by placing 10 grams of soil in a 125 ml Erlenmeyer flask containing 90 ml sterile distilled water heated to 40° C. The flasks were shaken, allowed to stand for 30 minutes, shaken again and allowed to settle. A second dilution of 1 : 8 was prepared by aseptically taking a 2 ml aliquot of the initial dilution from just beneath the surface of the liquid and placing this aliquot in a 30 ml screw-cap vial containing 14 ml sterile distilled water. Vials were shaken and allowed to stand

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[2]) A portion of this study was made possible through laboratory facilities provided by Dr. B. SAMANTARAI, Ravenshaw College, Cuttuck, Orissa, India.

for 10 minutes. This final dilution, representing a 1 : 80 dilution of the original soil sample, was sprayed with a sterile "Windex" ¹) atomizer onto the surface of a suitable agar medium contained in 60 mm diam. Petri dishes. This procedure was modified from that presented by SWATEK (1956).

In this isolation work Sabouraud's glucose agar medium was employed supplemented with penicillin, streptomycin and actidione in accordance with the procedure of GEORG et al. (1951). The medium was constituted as follows: 1 % peptone, 3 % syrupy glucose and 2 % agar. After autoclaving for 20 minutes at 15 lbs. pressure the medium was allowed to cool to about 50° C. At this time the antibiotics were added aseptically, mixed in well, and the plates were then poured immediately. The antibiotics were prepared as follows. Four grams actidione were dissolved in 20 ml 95 % ethanol and brought to 400 ml with sterile distilled water. Fifty ml of this solution were added per liter of medium. Ten ml sterile distilled water were added to a bottle containing one gram streptomycin, and one ml of this dilution was added per liter of medium. Five ml sterile distilled water were added to a bottle containing 500,000 units of penicillin, and ten ml of this dilution were added per liter of medium. The final medium is designated as Sabouraud's PSA agar.

This medium has the advantage that the Gymnoascaceae are usually not inhibited while the majority of Penicillia and Aspergilli are unable to develop. Thus, pure cultures of Gymnoascaceae may be obtained following a single transfer. Only in rare instances is one unable to obtain pure isolates.

In addition to the soil dilution procedure, a direct plating method was also used (ORR, 1957). For this 3 sterile 9 cm filter discs were placed in a sterile 90 mm diam. Petri dish and 60—90 grams of soil were placed on the discs. Sufficient sterile water was added to the soil to moisten it thoroughly and the dish was placed under a bell jar. Cultures which developed on these plates were transferred to plates of Sabouraud's PSA agar. Following development on the latter medium, transfers were made to plates and tubes of Freezing agar (ORR, 1959). Freezing agar is prepared in the following manner. Two-hundred grams of uncleaned, diced potatoes were infused with 500 ml tap water in a double-boiler top in the autoclave at 15 lbs. pressure for 15 minutes. The infusion was then mashed and filtered through a quadruple layer of gauze or cheese cloth and the volume was brought to one liter with distilled water. To this was added 20 g agar, 8 g dextrose, 0.5—1 g yeast extract and 0.5 g activated charcoal.

Isolations were made from dung by plating on Sabouraud's PSA agar. Subsequent transfers were made to Freezing agar on which stock cultures were maintained and frozen until desired for study. In other instances the material from dung was streaked on hay

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¹) Trade name.

infusion agar and then transferred to YpSs slants. The composition of YpSs medium is as follows: 1.5 % soluble starch, 0.4 % yeast extract, 0.1 % K_2HPO_4, 0.05 % $MgSO_4.7H_2O$ and 2 % agar.

Gymnoascaceae were isolated from rodent lungs by trapping the animals in snap traps and "Hava-hart" ¹) traps. The rodents were killed in the field with chloroform, followed by dissection and plating of the tissues. Those tissues which were plated included lungs, spleen, liver and heart. The organ was removed from the dissected animal with the aid of sterile scissors and scalpel. A small portion of each organ was partially buried in Sabouraud's PSA agar in Petri plates. Incubation was carried out at room temperature and at room conditions.

OBSERVATIONS

Pseudoarachniotus hyalinosporus sp. nov. ²)

Ascocarpiis discretis absentibus. Ascis aggregatis dispergentibus in hyphis sterilibus. Singulis ascorum aggregatis mensis 20—88 μ. diam., frequenter confluentibus ad majorem aggregatum. Ascis hyalinis, ovoideis vel obovatis, muris evanescentibus, 8-sporis, 5.5—8.8 × 7.7—11 μ, excludentibus pedunculatis mensis 2—5.5 × 2.2 μ. Ascosporis singulis hyalinis vel pallido-flavis, congregatis hyalinis vel pallido-flavis, ovatis vel oblatis, tenuibus muris levibus, (1.8) 2.2—2.3 (2.5) × 2.5—3.3 μ. Hyphis sterilibus fere muris tenuis, hyalinis, 1.7—2.2 μ diam., per occasionem magius hyphis diametri, 3.5—5 μ. muris cellularum crassioribus, paulum coloris fuscioris. Myceliis "racquet" presentibus, saepe formantibus erectos vel pedunculatis coremiformibus ad unum cm longis, saepe cum pigmento viridi. Sporis asexualibus fere absentibus; in stirpibus nonnullis arthrosporis hyalinis mensis 2—3 × 5—10 μ.

Discrete ascocarps absent. Asci in exposed groups scattered among the vegetative hyphae; individual groups of asci measure 20—88 μ diam., often confluent to form larger groups. Asci hyaline, ovoid to obovate, wall evanescent, 8-spored, 5.5—8.8 × 7.7—11μ, not including the stalk measuring 2—5.5 × 2.2 μ. Ascospores hyaline or pale yellow singly, hyaline to pale yellow en masse, ovate to oblate, thin-walled, smooth, (1.8) 2.2—2.3 (2.5) × 2.5—3.3 μ. Vegetative hyphae usually thin-walled, hyaline, 1.7—2.2 μ diam.; occasional hyphae of greater diam., 3.5—5 μ, with somewhat thicker cell walls which are slightly darker in color. Racquet mycelium present, often forming upright fascicles or coremiform stalks up to one cm long and often with green pigment. Asexual spore stage usually absent; in some strains hyaline arthrospores present, measuring 2—3 × 5—10 μ.

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¹) Trade name.

²) We wish to thank Dr. TIBOR BENEDEK for preparing the Latin description.

Colonies on YpSs at 28° C are pasty, submerged at first, with hyaline aerial mycelium developing within one week. The aerial growth usually becomes white and cottony, but may remain restricted. Within the white cottony areas there appear pale yellow tufts of hyphae which represent the asciferous areas. The appearance of the pale yellow color indicates that fruiting has been initiated. In about 3—4 weeks scattered green patches of hyphae may appear in the colony. Also, droplets of exudate may develop and are amber, yellow, colorless, or light to dark green. The reverse of the colony is usually pale lavender in 4 weeks, but in certain strains it is colorless, pale yellow or green. The pale lavender or green pigments diffuse into the medium to a greater or lesser extent. Certain strains form a profuse dark green pigment on the reverse side which colors the medium in one week old cultures and these strains are also very dark green throughout the surface of the colony. Strains which initially form a pale lavender pigment reverse usually develop green coloration reverse after about 60 days of incubation. However, there is much diversity of strain variation in this respect and some strains never form lavender or green pigments at any time. Certain strains form lavender but never green, others form green above but never on the reverse and other strains form pale yellow colors reverse while still others remain colorless reverse. However, the usual condition is for green above and reverse if incubation is continued for a sufficiently long period of time. In the young, pasty colony there often develop funiculose, erect, aerial strands of hyphae up to one cm high which persist throughout the incubation period. With the advent of fruiting each strand forms at its apex an apical cap of asciferous tissue which assumes the typical pale yellow color. In addition, asciferous hyphae may develop laterally throughout the length of the stalk.

Colonies on Freezing agar plus charcoal are pasty initially, developing white, yellow and finally green aerial growth. Reverse is yellowish to yellow-orange and there may be a rather dark central area in age which is almost brownish-orange. Pale yellow to slightly amber exudate may be present. Green coloration from the aerial hyphae may show through on the reverse side, but no green pigment colors the medium and a lavender color does not appear.

Colonies on Freezing agar minus charcoal are similar to the appearance with charcoal, but reverse in all strains is yellowish to brownish orange. There is some variation in the amount of yellow or green in the aerial colony. The amount of exudate is variable and it is usually amber in color but may occasionally be green.

The colonial appearance of a given strain of *P. hyalinosporus* on agar plates evidently varies greatly from one medium to another and in addition there are differences due to strain variation. Two of the strains, NRRL A9892, as well as the type strain, NRRL 2881, were cultivated on several additional media in order to ascertain the approximate range of variation on these media. The following descriptions pertain to these 2 strains.

Colonies on Sabouraud's maltose agar are pasty at first, then restricted white aerial hyphae develop in older areas. The thin colony is tinged red-brown due to the rust color of the reverse side. Above and reverse sides of the colony are furrowed. In 15 days colonies have a white, cottony, central region with radial extensions of the white cottony growth reaching to the margins. Alternating with the radial extensions of white cottony growth are areas of rust-colored, restricted hyphae. From the reverse side of the colony the appearance is that of a woody dicotyledonous stem resulting from the alternating colored hyphal regions. After 30 days incubation a greenish coloration is quite pronounced throughout the colony.

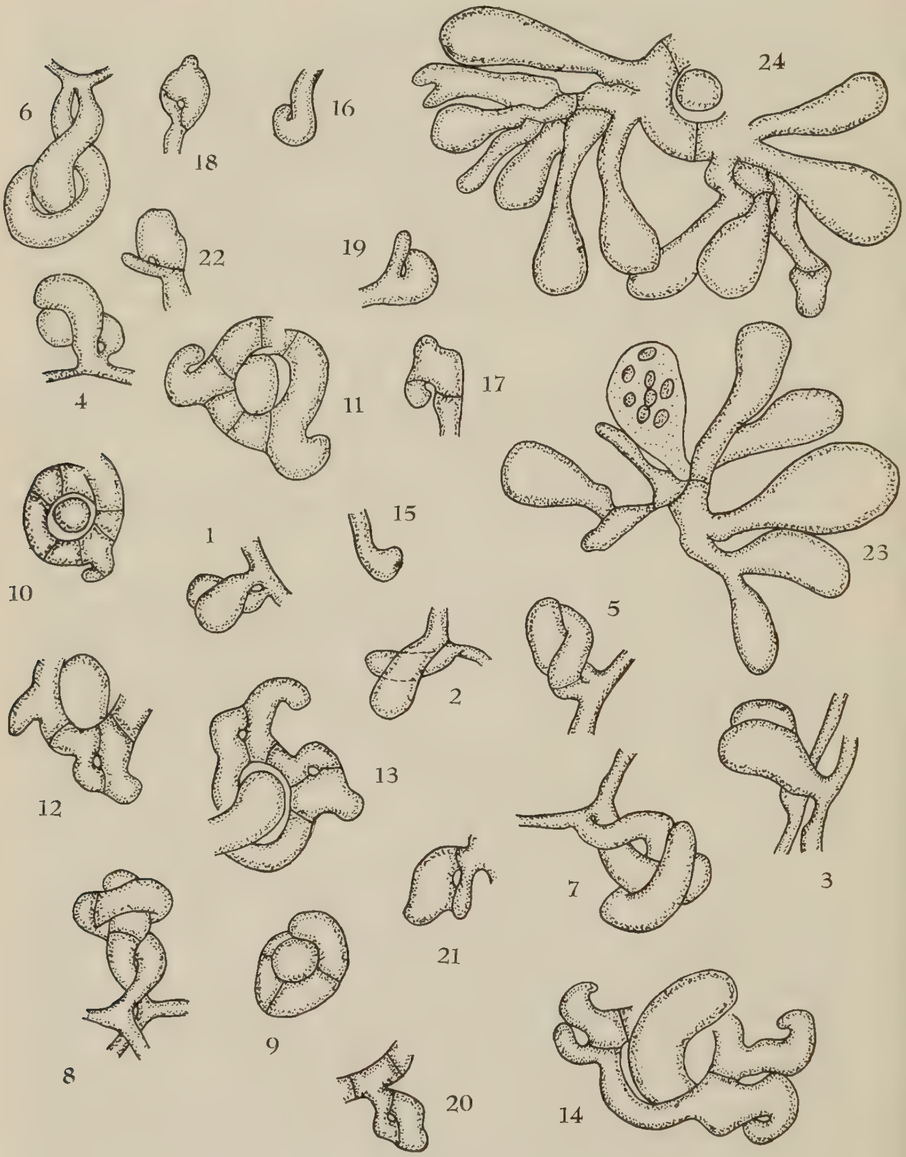
Colonies on Czapek's agar are not thin as is usually true of Gymnoascaceous fungi, but instead are white and cottony. A colorless exudate is formed. The reverse is creamy-pink to dull orange. Ascogenous areas develop after 30 days and are evident as white tufts of hyphae within the yellowish hyphal areas.

Colonies on malt extract agar are pasty initially and never develop aerial hyphae. Reverse is colorless. No asci are formed on this medium.

Since many mycologists might prefer to identify the Gymnoascaceae without resorting to the isolation of these slow-growing fungi in pure culture, it would be convenient to describe the characteristics of one strain of *P. hyalinosporus*, the type strain, NRRL 2881, as it appeared on the original dung material from which it was isolated.

Tufts of mycelium were white at first, then tinged with yellow. Many clusters of asci were nestled at random in the hyphal tufts. These clusters soon lost their discrete nature and became one large confluent group of ascospores. Asci were ovoid, 6.6—8.8 × 9.9—11 μ. Ascospores were hyaline, ovate to oblate, smooth, thin-walled, 2.2—2.5 × 3.3 μ. Vegetative hyphae measured 1.5—2.2 μ diam. Ascus clusters varied from very small, with 4 asci, to 70 μ diam., with many asci. No asexual spores were found, although in pure culture this strain produced arthrospores.

The formation of gametangial initials and their subsequent development to produce asci was studied. The gametangial initials arise as adjacent lateral branches from the same (Fig. 1, 2, 4) or different (Fig. 3) parent hyphae. The two initials are very closely appressed to one another so that at times it may appear as though only one club-like structure is present. As they continue further development the apical portion of one gametangium, the ascogonium, forms a loop at right angles to the plane of the other, central initial thereby presenting a very characteristic appearance (Fig. 6, 7, 8). A few septa are formed either early (Fig. 5) or late (Fig. 8. 9) dividing the gametangia into several cells. Ascogenous hyphae develop from some of the ascogonial cells (Fig. 10) and these hyphae soon recurve to form croziers (Fig. 11, 12, 14). The first formed croziers usually do

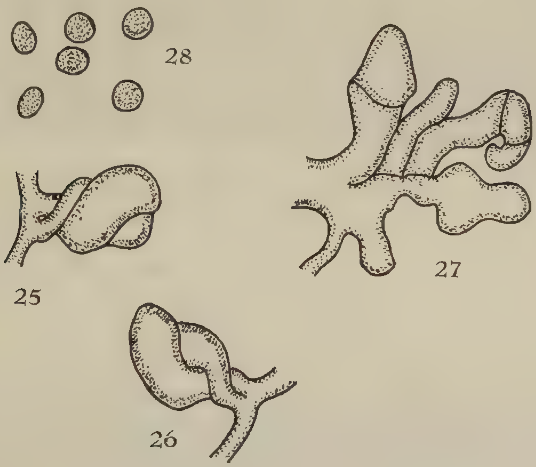


Figures 1—24. *Pseudoarachniotus hyalinosporus.*

Fig. 1—4. Young gametangial initials. 5. Initials become septate and coil tightly about one another. 6—8. The apical portion of one initial forms a distinct loop around the other initial. 9. Initials as seen from above, showing the central initial with the other initial forming a loop about it. 10—11. Ascogenous hyphae appearing from cells of the loop. 12—14. Croziersformed from short ascogenous hyphae, and the formation of secondary croziers. 15—22. Stages in formation of croziers and secondary croziers. 23—24. A portion of the group of asci developing from a single coil. × 1455.

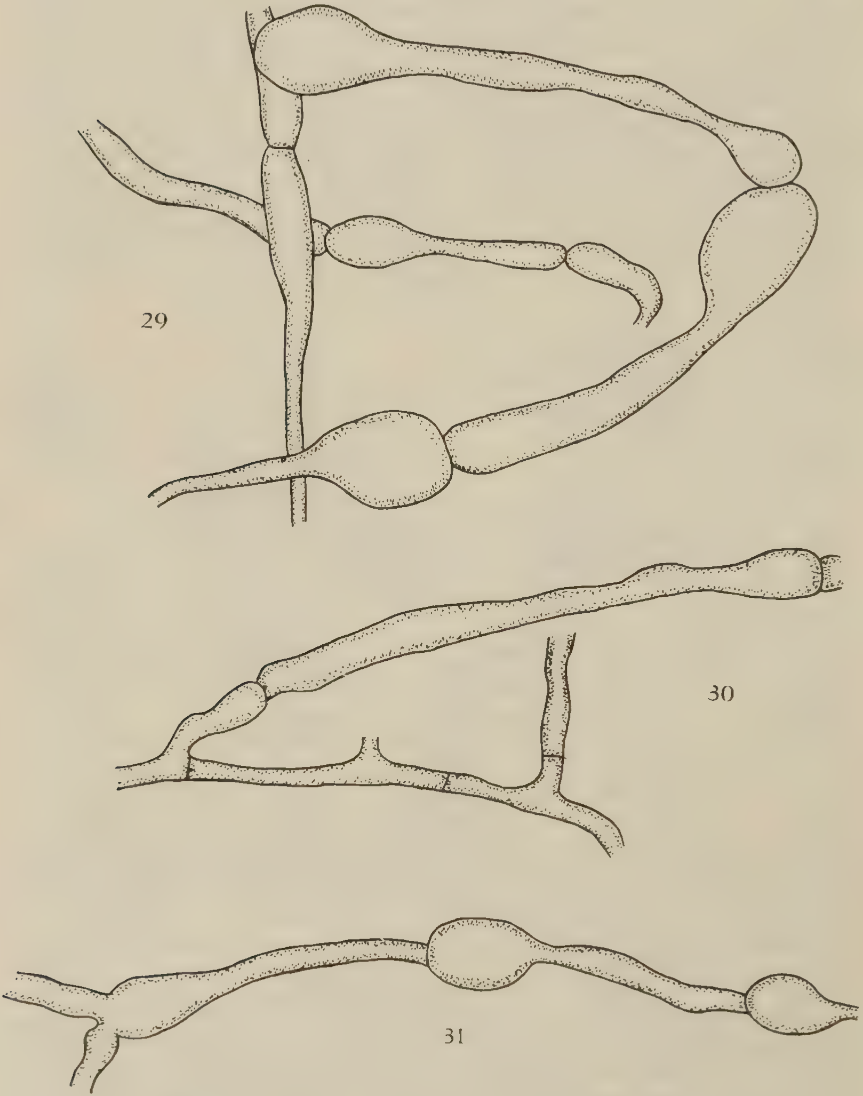
not form asci but rather grow out to produce additional croziers (Fig. 12, 13) which then form asci. In this manner many asci ultimately result from one original pair of gametangia (Fig. 23, 24).

In most of the strains of *P. hyalinosporus* there are large hyphal strands of racquet mycelium, often with darker pigmented cell walls, which lie in and around the ascus clusters and appear almost to aggregate them or hold them together to form a simple ascocarp. However, these hyphal strands are scattered at random throughout the colony, and there is no relation between their position and that of the ascus clusters. Hence, no true ascocarp is formed One strain in particular, 0—874, has a great many of these hyphae, but it has other characteristics which clearly reveal its true taxonomic position.



Figures 25—27. *Pseudoarachniotus hyalinosporus*.
Fig. 25—26. Young initials showing how closely adherent they may be. 27. A portion of the system of croziers and asci formed from part of the coil. 28. Mature ascospores. × 1727.

Of the 54 strains of *P. hyalinosporus*, 25 were isolated from lungs of rodents, 9 were from various sorts of dung, 19 were from soil and one was isolated from a lesion, a tinea pedis condition, on the foot of a human. Two strains were isolated in Muscatine, Iowa, in 1959, from material collected in Cuttuck, Orissa, India. The type strain, NRRL 2881, was isolated from guinea pig dung, while the second strain, NRRL A-9892, was isolated from rabbit dung. All other strains were isolated in California from materials collected within that state. The culture numbers listed here refer to accession numbers of G. F. Orr.



Figures 29—31. *Pseudoarachniotus hyalinosporus.*
These depict a portion of the system of stouter strands of racquet and other hyphae which grow throughout the colony and which therefore may be found in the vicinity of the groups of asci. × 1727.

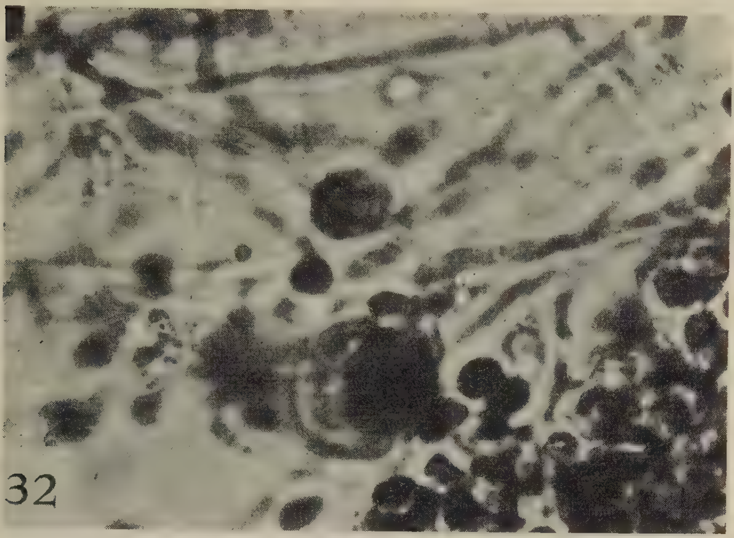


Fig. 32 A gametangial coil showing how initials may arise from the same parent hypha. × 1818.

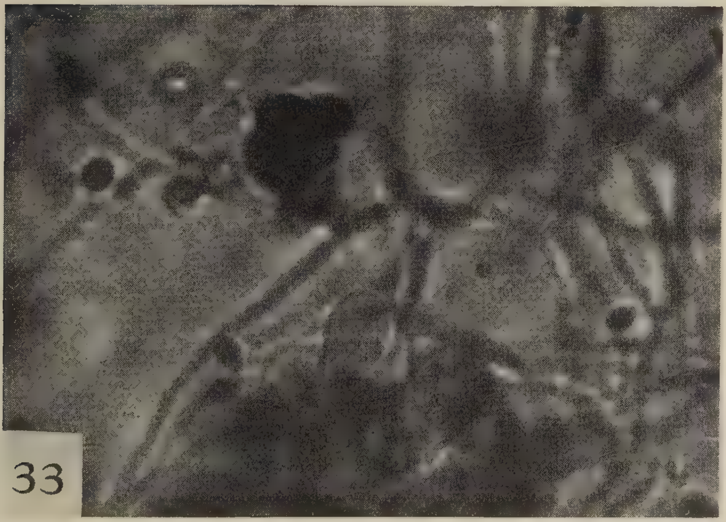


Fig. 33. A coil, some immature asci and some free ascospores. × 1667.

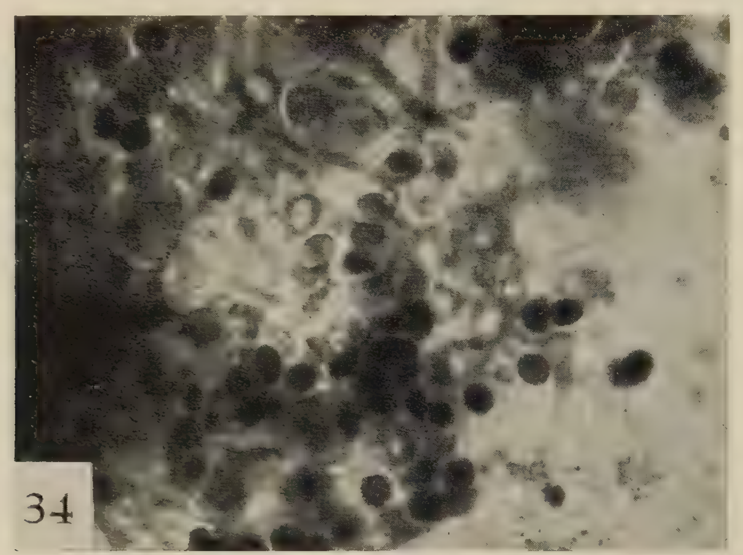


Fig. 34. Ascospores, asci and large racquet hyphae. × 1515.

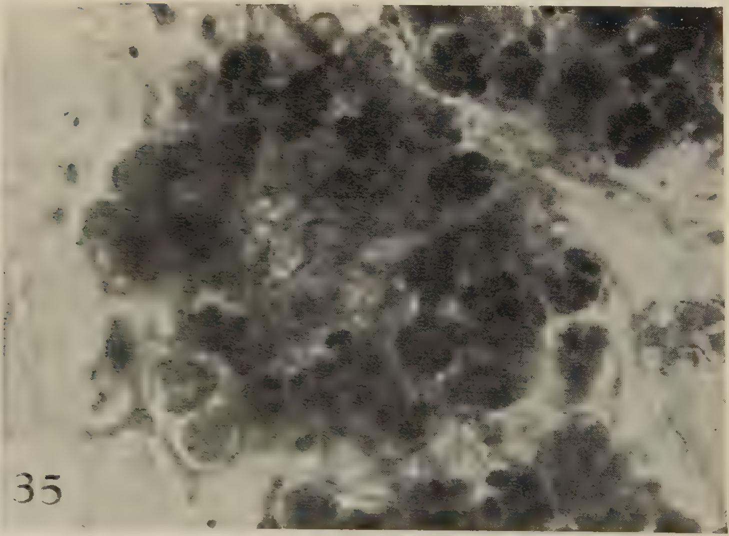


Fig. 35. Mature asci containing ascospores. A coil may be distinguished amid the asci. × 1212.

Those strains of *P. hyalinosporus* which were isolated from lungs of the kangaroo rat (*Dipydomys* sp.) include: 0—270, 12 miles southwest of Inyokern, Kern County; 0—486, rat # 19, Blythe Air Strip, 6 miles west of Blythe, Riverside Co.; 0—699, rat # 10, Blythe Air Strip; 0—756, 4 miles southwest of Palo Verde, Imperial Co.; 0—783, Palmdale, Los Angeles Co.; 0—840, rat # 1, Blythe Air Strip; 0—841, rat # 2, Blythe Air Strip; 0—842, rat # 3 Blythe Air Strip; 0—843, rat # 4, Blythe Air Strip; 0—844, rat # 5 Blythe Air Strip; 0—846, rat # 6, Blythe Air Strip; 0—847, rat # 7, Blythe Air Strip; 0—848, rat # 8, Blythe Air Strip; 0—849, rat # 9 Blythe Air Strip; 0—850, rat # 11, Blythe Air Strip; 0—851, rat # 12, Blythe Air Strip; 0—852, rat # 13, Blythe Air Strip; 0—853, rat # 15, Blythe Air Strip; 0—854, rat # 16, Blythe Air Strip; 0—855, rat # 18, Blythe Air Strip; 0—856, rat # 20, Blythe Air Strip; 0—857, rat # 21, Blythe Air Strip; 0—878, rat # 5, Blythe Air Strip. Strains from lungs of the pocket mouse include: 0—862, mouse # 6, 8 miles east of Indio, Riverside Co.; 0—863, mouse # 7, 8 miles east of Indio.

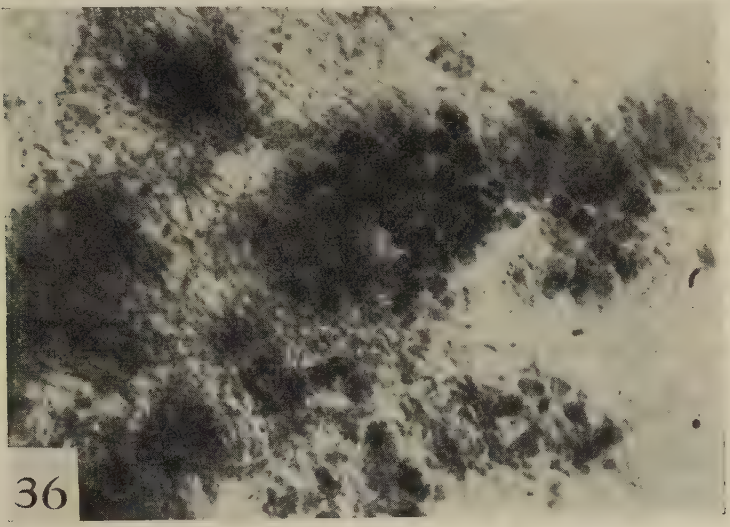

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Fig. 36. Separate groups of asci arising from different gametangial coils. × 455.

Strains of *P. hyalinosporus* isolated from soil include: 0—66, 14 miles southwest of Inyokern, Kern Co., 0—103, Lancaster, Los Angeles Co.; 0—105, 12 miles southwest of Inyokern; 0—145, 12 miles southwest of Inyokern; 0—215, 12 miles southwest of Inyokern; 0—268, north of Lancaster; 0—276, 14 miles southwest of Inyokern; 0—286, 5 miles southwest of Palo Verde, Imperial Co.; 0—292, miles south of La Grange, Stanislaus Co.; 0—314, 12 miles southwest of Inyokern; 0—698, soil sample # 1, Blythe Air Strip; 0—743, 6 miles southwest of Palo Verde; 0—760, north of Lancaster; 0—767, 14 miles southwest of Inyokern; 0—858, soil sample # 1, Blythe Air Strip; 0—859, soil sample # 2, Blythe Air Strip; 0—860, soil sample # 3, Blythe Air Strip; 0—861, soil sample # 4, Blythe Air Strip; 0—864, 6 miles southwest of Palo Verde.

In addition to strains NRRL 2881 and A-9892, several other strains were isolated from dung, including: 0—110, fox dung, 16 miles southwest of Inyokern, Kern Co.; 0—299, skunk dung, 23 miles east south east of the junction of highways 41 and 466, Kern Co.; 0—311, fox dung, 13 miles southwest of Inyokern; 0—865, fox dung, 13 miles southwest of Inyokern; 0—874, rat dung, Lake Arrowhead area, San Bernardino Co.; 0—876, sheep dung, Oildale, Kern Co.; 0—911, fox dung, Lake Arrowhead area.

One of us (GFO) received a fungus from Dr. J. WALTER WILSON which had been isolated from a tinea pedis lesion on the foot of a human. This fungal culture yielded two distinct fungi, one was *Trichophyton rubrum* and the other proved to be a strain of *P. hyalinosporus* (0—877). Thus, another representative of the Gymnoascaceae may be added to the list of those isolated from pathogenic circumstances adding fuel to the argument for the supposed relationship between dermatophytes and Gymnoascaceae. In this connection, it is interesting to note that of 35 strains of *P. hyalinosporus* which were tested, all grew and fruited more rapidly at 37C than at 28C.

A few additional comments appear to be in order regarding strain variation in *P. hyalinosporus*. Certain strains growing side by side on YpSs agar might appear so different as to be considered separate species. For example, strains 0—760, 0—292, 0—276 and NRRL 2881 develop an intense green color in the mycelium and reverse within a few days after inoculation, while strain 0—105 is colorless reverse and 0—743 has a yellow reverse. In strains 0—852, 0—855, 0—857, 0—862 and 0—853 the colonies are pale yellow, completely devoid of any green coloration and even after 60 days the reverse is pale lavender with no green pigment.

It is conceivable that strains from lungs might differ culturally from strains isolated from soil and or dung. However, there was no consistent relation of colonial pattern to habitat in nature. Those cultures developing heavy green pigmentation early were either from soil (0—268, 0—276, 0—292, 0—760) or from dung (NRRL 2881). Those strains which at first were pale lavender reverse but green tardily were from lungs (0—270, 0—486, 0—699, 0—840, 0—841, 0—842, 0—844, 0—846, 0—848, 0—849, 0—850, 0—851), soil (0—215, 0—314, 0—698, 0—858, 0—859, 0—861) or dung (0—110, 0—299, 0—311, 0—865). Strains which never formed even a trace of green reverse but which produced green in the mycelium included some from lungs (0—783, 0—847, 0—856, 0—863) and from soil (0—103, 0—105, 0—743, 0—860). Another group, all from lungs, did not develop green either in the mycelium or on the reverse (0—852, 0—853, 0—855, 0—857, 0—862). Strains 0—110, 0—311 and 0—865 displayed an additional colonial characteristic which was absent in all other strains. These 3 isolates produced peculiar shiny-white, tortuously branched growths which developed above the aerial mycelium and were quite similar to the habit of the myxomycete, *Ceratiomyxa*. It is very interesting that these 3 strains were found on fox dung from two different localities. Strain 0—911, also from fox dung, did not develop these white branched structures.

All strains, regardless of type of colonial coloration or growth pattern, are identical in two characteristics. All have the same type of ascospore—thin-walled, small, hyaline to pale yellow, smooth, and measuring about 2.2 × 3.3 μ. Furthermore, the appearance of the initials in the early stages of development is a characteristic one in *P. hyalinosporus* and has not been detected in other species of the family. The young stage of the initials often appears as if there is one club-shaped structure, whereas in actuality there are two initials present and so closely adherent as to make them appear as one. This character may be used for taxonomic purposes since initials are plentiful and easily seen.

A glance at the list of localities from which the strains of *P. hyalinosporus* were isolated would suffice to permit the conclusion that this species is indeed widely distributed. Strains were found on two continents on opposite sides of the globe. Numerous strains were found in each of two rather widely separated desert regions in California, the Colorado (Indio, Palo Verde) and the Mojave (Palmdale, Inyokern) deserts. It might be thought odd that with such a distribution this species had not been reported previously. However, it is not too surprising in light of two considerations: the slow growth of the Gymnoascaceae in general and the absence of a specific isolation procedure for these fungi.

Isolation of *P. hyalinosporus* from lungs of rodents is another feature worthy of note. Out of 27 kangaroo rats which were examined in the Blythe area, 19 yielded *P. hyalinosporus* when the lungs were plated. However, there is no reason to consider this species to be pathogenic, since spores in the sandy desert soil might easily be inhaled by rodents as they burrow in the soil. EMMONS (1954) reported the isolation of *Gymnoascus* (*G. brevisetosus*, KUEHN, 1956) and *Myxotrichum* from rodent lungs. Therefore, it would seem that at least certain Gymnoascaceae have an affinity for rodent lungs, but this entire problem area is in need of experimental consideration.

Previously described species of *Pseudoarachniotus* include *P. roseus* KUEHN, *P. citrinus* KUEHN (1957) and *P. reticulatus* KUEHN and GOOS (1960). These 3 species may be distinguished from *P. hyalinosporus* with comparative ease. *Pseudoarachniotus reticulatus* has globose, echinulate-reticulate ascospores while the other three species have smooth, oval to oblate ascospores. The ascospores of *P. roseus* and *P. citrinus* are large, over 3 μ diam., longer than 4 μ, and thick-walled, whereas the ascospores of *P. hyalinosporus* are small, about 2,2 × 3.3 μ, and thin-walled. The colonial coloration is an added diagnostic feature; colonies are red in *P. roseus*, yellow in *P. citrinus* and white to pale yellow and usually with some green in *P. hyalinosporus*.

We would like to express our sincere appreciation to Dr. O. A. Plunkett and Dr. F. E. Swatek who collected many of the dung and soil specimens and who also performed the task of plating the lungs of the rodents autopsied in the field.

Summary

A new species of *Pseudoarachniotus*, *P. hyalinosporus*, is described and illustrated. A total of 54 isolates of this species was studied and their growth was compared on several media.

Although certain differences appeared in colonial coloration and growth characteristics, all strains were similar in 2 fundamental characteristics, viz., type of ascospore and type of gametangial initials and their subsequent development. The initials in early stages are often so closely appressed one to another that they give the appearance of a single structure. Further development takes place by the elongation of the ascogonium which forms a loop around the male initial at right angles to the linear plane of that initial. The ascospores of *P. hyalinosporus* are small, about 2.2 × 3.3 μ, thin-walled, oval, hyaline to very pale yellow, and smooth, thereby differing from *Pseudoarachniotus reticulatus* which has globose, echinulate-reticulate ascospores, and *P. roseus* and *P. citrinus* which have large, thick-walled, oval to oblate ascospores. Furthermore, the colonies of *P. roseus* are red, those of *P. citrinus* are yellow, and in *P. hyalinosporus* colonies are white with pale yellow asciferous areas and usually with a great deal of green pigment both above and reverse. Of the 54 strains of this new species, 25 were isolated from rodent lungs, 9 from dung, 19 from soil and one from a tinea pedis condition. *Pseudoarachniotus hyalinosporus* was found in two collections from India and it appears to be widely distributed in California.

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MECHANISM OF FUNGAL PATHOGENICITY

A round table discussion, held during the 61st Annual Meeting of the American Society for Microbiology, Chicago, Ill. April 25,1961

At the initiative of the Chicago Medical Mycological Society, through its president, John W. Rippon, a round table discussion was organized, during which eight short, invited papers were presented. The meeting was well attended (standing room only!) and at the end of the presentations a lively discussion followed. Under the leadership of the able moderator, Esther Meyer, the program had a full success. A condensed report of the events, based on the abstracts of the authors, here follows.

1) St. Marcus, G. A. Hill and R. A. Knight reported on the

Induction of Systemic Mycotic Infection in Laboratory Animals

A previously described (Bact. Proc. p. 88, 1952) standardized and reproducible method for the measurement of mouse virulence (lethality or infection) of systemic mycotic organisms was discussed in the light of subsequent work. The method has yielded interesting information such as the following:

1. Intravenously injected graded doses of yeast phase organisms of strains of *H. capsulatum*, *B. dermatitidis*, *Cr. neoformans*, and *C. albicans* result in mortality related to challenge dose; mice that survive an LD_{50} dose are invariably infected.
2. As long as the organisms are kept at 37° C and subcultured at weekly to monthly intervals, the population will show stable virulence characteristics.
3. Storage of slants at refrigerator temperature may result in depression of virulence.
4. Repeated (15 times) mouse passage may or may not enhance the virulence of different strains of these organisms.
5. Marked variations exist in virulence of strains of these organisms. We have studied particularly 2 strains of *H. capsulatum* and 2 strains of *B. dermatitidis* which are markedly different in virulence (*H. capsulatum*: strain G17M—$LD_{50} =$ ca. 10^6, and of 6651 $= 5 \times 10^6$; *B. dermatitidis*: strain 410—$LD_{50} = 32$ to 406 organisms and of strain 380 $= >10^6$).
6. Intraperitoneal or subcutaneous injection of vaccines prepared as formalin killed yeast phase suspensions protects against intravenous or subcutaneous challenge.

7. The degree of resistance engendered by chronic infection with *H. capsulatum* was not observably greater than that induced by immunization.

8. Resistance following infection or immunization does not appear to depend primarily on humoral factors but rather on changes induced in capacity for phagocytic cells to destroy ingested organisms.

9. Mice are readily immunized against infection with polysaccharides derived from *H. capsulatum.*

How real, in terms of human affliction, these results may be cannot be answered. We do know that mouse virulence does not reflect guinea pig virulence. We have found difficulty in inducing progressive histoplasmosis in guinea pigs and failed completely with cynomolgous (*Macacus irus*) monkeys, although others have successfully infected *M. rhesus* and *M. mulatta.* Assessment of significance of results obtained with mouse experiments remains open.

⁂

2) JAN SCHWARZ discussed

The Dog as Index of Endemicity of Mycoses.

EMMONS suggested that the infection rate of dogs in an endemic area of Histoplasmosis could be taken very well as an index of the prevalence of disease. When applied in Cincinnati, which is in the endemic area of histoplasmosis, it was found that in a group of 100 dogs with morphologic methods, 20 were found to have residuals of histoplasmosis. In a subsequent study 132 stray dogs were examined and combining morphologic and cultural techniques, 39 % were found to be positive. Similarly in Tucson, Arizona, which is in the endemic area of coccidioidomycosis, in 194 dogs, 26 % of positivity was obtained. The explanation why dogs which are clinically healthy are carriers of virulent organisms is hard to give.

⁂

3) C. W. EMMONS spoke about

Isolation of Histoplasma Capsulatum from Soil in Washington, D. C.

Reported isolations from soil of *Histoplasma capsulatum* have been generally from specimens collected in rural areas. In some cases of urban human histoplasmosis the patients had an exposure to manure, guano, or top soil imported from rural areas, and a rural source of infection has been generally sought or assumed. Exposure to composted chicken manure has been the most frequent association noted.

Isolation of *H. capsulatum* has been reported from a school yard and from a large neglected park overgrown with bushes and vines. Its growth in both these areas was associated with the presence of starlings. Results of skin testing have suggested that there may be frequent urban exposure to *H. capsulatum.* It has been suggested that the house bat, *Eptesicus fuscus* may be a factor in permitting growth of the fungus near urban dwellings.

H. capsulatum was isolated, in the investigation reported here, from 10 of 10 specimens of soil collected in a small park adjacent to Pennsylvania Avenue in downtown Washington. This park contains several small sycamore trees which are roosting sites of starlings. The fungus was isolated also from one of five soil specimens collected under oak trees near Massachusetts Avenue and E Street, N. E., in Washington.

The public health aspects of these first isolations of *H. capsulatum* from a busy shopping area of a large city require further investigation. Virulent strains of *H. capsulatum* and *Cryptococcus neoformans* are now known to occur in congested urban areas. ¹).

⁂

4) S. B. Salvin and R. F. Smith reported on
Delayed Hypersensitivity and Cryptococcosis.

Sensitization of guinea pigs with minute quantities of highly purified soluble proteins has resulted in delayed hypersensitivity followed in time by Arthus type hypersensitivity and by detectable circulating antibody. From such experiments conclusions were drawn (a) that the specificity of delayed hypersensitivity is directed toward broad, basic portions of the antigen molecule, whereas the specificity of circulating antibody is directed toward smaller, terminal areas of the antigen, and (b) that delayed hypersensitivity may be an intermediate phase in the immune process leading to the formation of circulating antibody. Polysaccharide from *Cryptococcus neoformans* may be considered to act as a hapten, and therefore can combine with antibody in serologic tests but is not active in inducing or detecting delayed hypersensitivity. Protein preparations from *C. neoformans* on the contrary can induce delayed hypersensitivity in guinea pigs and are active for the detection of delayed hypersensitivity in experimentally infected guinea pigs. This preparation did not cross-react in skin reactions in guinea pigs infected with *Histoplasma capsulatum, Coccidioides immitis*, and *Candida albicans*. However, protein preparations obtained from different isolates within the species of *C. neoformans* did cross-react.

⁂

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¹) This report will appear in full in Public Health Reports.

5) M. Huppert discussed the

Role of Hypersensitivity in Pathogenesis of Coccidioidomycosis.

Empirical observations indicate that, in human cases of coccidioidomycosis, delayed-type hypersensitivity is associated constantly with resistance to the spread of the infection. More than 99 % of the clinical non-disseminated cases yield a positive skin test, whereas only 30 % of cases with disseminated disease are skin test positive. The incidence in the latter instance can be increased to 60 % positive when the concentration of the skin test material, coccidioidin, is increased tenfold. In the disseminated disease cases, in whom a positive skin test has been maintained, there has been a 75 % survival rate, but this has been reduced to 20 % in those patients with a negative skin test. It appears, therefore, that persistent hypersensitivity parallels resistance to spread of the infection and is indicative of a favorable outcome, but that loss of hypersensitivity parallels progression of the disease and has an unfavorable prognosis.

There is no direct experimental evidence to establish whether this delayed-type hypersensitivity is the cause of resistance to spread of the infection or merely develops independently, but parallel, to such resistance. On the basis of studies which have been reported separately by Uhr, Lawrence, Pappenheimer, Salvin, Chase, and Gell, the author proposed a hypothesis to explain the relationship between hypersensitivity and resistance to spread of infection in coccidioidomycosis. The conclusion reached was that delayed type hypersensitivity functions initially as a host defense mechanism, but that it contains a tissue destructive component which can be dangerous to the host in the presence of excess antigen and serum antibody.

. ˙ .

6) D. B. Louria reported on

The Pathogenesis of Experimental Infections due to Cryptococcus and Candida.

Host defenses against Cryptococcal infection are generally considered to be inadequate. Data were presented to show that defense mechanisms in the mouse are in actuality quite good. Immunization with living Cryptococci provided protection against subsequent challenge with ordinarily lethal inocula. Infection with small numbers of *C. neoformans* was associated with progressive increase in fungus census within all tissues during the initial 7 to 14 days after inoculation. Thereafter tissue populations in the majority of animals fell progressively so that tissues were virtually sterile 3 to 6 months after the onset of infection. Mice challenged with lethal inocula at this time demonstrated marked prolongation of life and increase in survivorship. The studies suggested that defenses against *Cryptococcus* are antibody dependent and maybe expected to be generally adequate for control of the parasite. They also suggest that the localization of infection to the central nervous system in previously healthy humans and the widespread extra-renal involvement observed in persons with malignant disease of the reticulo-endothelial system may be ascribed to the presence of suboptimum amounts of antibody crossing into the central nervous system in the former instance and to the inadequacy of host immune responses in the latter.

In animals infected with *C. albicans* progressive disease is limited to a single organ, the kidney. Serial histologic studies suggested that the renal localization was related to the ability of the fungus to rupture into and grow within the renal tubular lumen, a site in which the fungus appears to be protected from host cellular defense mechanisms. Such renal localization was observed with strains of different virulence. The more virulent strains differed from the less virulent strains in their ability to produce rapid death in the absence of microbial multiplication presumably by production of exotoxins or endotoxins. Furthermore, the more virulent strains show a striking ability to grow out of viable polymorphonuclear leukocytes following phagocytosis. It is suggested that this ability to form mycelia within host cells contributes to toxicity and/or to renal parasitism and represents an important biologic phenomenon in the pathogenesis of candidiasis.

⁂

7) H. F. Hasenclever and W. O. Mitchell spoke about

Antigenic Studies with Candida.

III. Comparative pathogenicity of C. albicans Group A, Group B, and C. stellatoidea.

Antigenic groups have been observed and reported in *Candida albicans*. Further investigation indicated the antigenic similarity of *C. albicans* Group A with *C. tropicalis* and *C. albicans* Group B with *C. stellatoidea*. This investigation was undertaken to determine if any differences in virulence could be detected between strains of *C. albicans* Group A and *C. albicans* Group B strains. *C. stellatoidea* strains were also included in the study. Similar experiments with *C. tropicalis* have been reported.

Twenty-four strains of *C. albicans* Group A, 23 strains of *C. albicans* Group B, and 11 strains of *C. stellatoidea* were studied with respect to mouse virulence. Five *C. albicans* Group B strains and five *C. stellatoidea* strains were tested for lethality in rabbits.

The virulence for mice of both antigenic groups of *C. albicans* was approximately the same. Although *C. stellatoidea* was generally less virulent for mice than *C. albicans*, some strains were more virulent for mice than the least virulent *C. albicans* strains. All five *C. albicans* Group B strains tested in rabbits demonstrated considerably more virulence for this laboratory animal than any of the four *C. stellatoidea* strains tested. [1]).

. . .

8) F. J. ROTH discussed the

Inhibition of Growth of Pathogenic Yeasts by Human Serum.

The serum of normal adults possesses marked capacity to inhibit the *in vitro* growth of *Candida albicans*. In patients with acute blood dyscrasias this inhibitory factor is greatly diminished. Sera from patients with Hodgkin's disease, multiple myeloma, acute leukemia, and erythemic myelosis are especially low in titer. No such reduction is observed in the serum of individuals with cutaneous moniliasis, diabetes, systemic mycoses, and metastatic carcinomas. The growth of all species of *Candida* associated with man are inhibited to some degree by normal serum; *C. albicans* appears to be the most resistant to this growth retarding mechanism. The inhibitory action is not associated with classical antibodies as absorption with diverse forms of homologous and heterologous antigen preparations fail to influence the activity of treated serum. The activity of serum is independent of carbohydrate concentration and is only moderately affected by dialysis. The inhibitory capacity of normal serum is little effected by exposure to 56° C but is largely destroyed by heating at 60° C. for 1—2 hours. The utilization of a variety of nitrogen sources and increased dextrose and vitamin concentrations fail to significantly diminish the inhibitory activity of sera as measured in the assay procedure. Serum from subjects receiving oral therapeutic doses of tetracyclines for 12 days or more failed to show significant decrease in ability to inhibit the growth of *C. albicans*. The interdependent role of trace elements, vitamins, and serum proteins in the total inhibitory mechanism suggests a complex of operative factors in the *in vitro* situation. The role of the inhibitory factors in the pathogenesis of candidiasis is under investigation.

. . .

The invited speakers presented a wealth of excellent material of their own current research. The Chicago Medical Mycological Society deserves great credit for the succesful arrangement of this round table discussion.

TIBOR BENEDEK

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[1]) A complete report of this investigation will appear in the Journal of Bacteriology.

The Participants of the Round Table

1) Dr. Stanley Marcus, Professor, Department of Bacteriology, University of Utah, College of Medicine, Salt Lake City, Utah.

2) Dr. Jan Schwarz, Department of Pathology and Clinical Laboratories of the Jewish Hospital of Cincinnati, Ohio.

3) Dr. Chester W. Emmons, Chief, Medical Mycology Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Md.

4) Dr. S. B. Salvin, Scientist Director of the National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton, Montana.

5) Dr. Milton Huppert, Chief, Mycology Research Laboratory, Veterans Administration Hospital, San Fernando, Calif.

6) Dr. Donald B. Louria, Head, Infectious Disease Laboratory, Bellevue Hospital, Second (Cornell) Medical Division, New York, N. Y.

7) Dr. H. F. Hasenclever, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Md.

8) Dr. Frank Roth, Associate Professor of Dermatology and Microbiology, University of Miami, School of Medicine, Coral Gables, Florida.

CORRADO CAPRETTI
(1915 - 1960)

OBITUARY

CORRADO CAPRETTI

(1915—1960)

El 13 de diciembre de 1960 falleció el Dr. CORRADO CAPRETTI, Profesor de Micología de la Universidad de Los Andes, Mérida/Venezuela, a consecuencia de una infección contraida mientras trabajaba en su laboratorio haciendo estudios en *Histoplasma capsulatum*. Gracias a minuciosos estudios de investigación que le ocuparon más de un año y al mismo tiempo le costaron la vida, logró el Dr. CAPRETTI comprobar esta especie fungina en el suelo de varios sitios de la Cuenca del Lago de Maracaibo. Así contribuyó esta ilustre victima de la ciencia con un aporte valioso a los conocimientos nosológicos y epidemiológicos de nuestro pais, estableciendo sólidas bases científicas para las futuras luchas sanitarias contra éste hongo patógeno, algunas veces mortal.

Nacido el 13-4-1915 en Firenze/Italia, adquirió los primeros conocimientos de trabajo en laboratorios durante su tiempo de prisionero de guerra en un Hospital Militar de la India.

Regresó a Italia y trabajando como técnico de laboratorio logró estudiar bachillerato y después graduarse con altas calificaciones en la Facultad Agraria de la Universidad de Firenze. En el mismo Instituto de Patología Vegetale (Director Prof. BIRAGHI), donde había trabajado come técnico, desempeñó luego el cargo de asistente universitario.

Contratado como Profesor de Micología por la Universidad de Los Andes, el 1-10-56, fundó y organizó su laboratorio entre nosotros, trabajó activamente en el campo de la docencia en varias Facultades, en el campo del diagnóstico de enfermedades funginas y en la investigación prestando su valiosa colaboración a diferentes cátedras y servicios asistenciales afines.

Dotado de una voluntad extraordinaria de trabajo y de un espiritu de superación logró publicar varios trabajos científicos y contribuir activamente en varios Congresos y Jornadas Científicas del pais. Dos veces salió al exterior, a Chamblee/Atlanta, Georgia, EE.UU. y a Recife-Brasil, para tomar cursos de Micología y profundizar sus conocimientos en la materia.

Fuera de su dedicación continua a los estudios científicos en los cuales se distinguió por su energía de organizar, su manera de trabajar en forma seria, sin alardes de ninguna especie y la modestia propia de todos los hombres valiosos, era un padre de familia ejemplar, vivía una vida sencilla y modesta en el seno de su hogar, siendo un ferviente católico.

Quién escribe estas lineas, há perdido un fiel y sincero amigo personal y un valioso y apreciado colaborador con quién llevó a cabo una fructifera labor conjunta en la cual se complementaron los estudios anatomopatológicos con estudios micológicos.

KARLHANNS SALFELDER

LIBRI NOVI

Developments in Industrial Microbiology. Vol. I. Proc. of the 16th General Meeting of the Society for Industrial Microbiology. Plenum Press, New York, 1960, 267 pp.

This book is a "record of the symposia, special lectures and contributed papers presented at the Sixteenth General Meeting of the Society for Industrial Microbiology", August 30-Sept. 3, 1959. It is planned to issue such a volume annually to cover similar meetings, and it is the opinion of this reviewer that such a project is indeed worthwhile. Volumes such as these will fill the void left by the material found in the several books on Industrial Microbiology whose contents are long out-dated by the time they appear in print. It will now be possible for those interested in the industrial applications of microbiology to keep abreast in the problem areas as they change and develop from year to year in this rapidly expanding scientific endeavor.

Although the contents of this volume were not intended to present a resume of the current fields of activity within Industrial Microbiology, nevertheless, a glance at the included topics reveals the broad scope of this branch of science and the overlapping of classic interdisciplinary boundaries. Topics include a discussion of academic training for careers in industrial microbiology; the part that microbiologists play in the conquest of space, including a discussion of germ-free mammals, and the use of algae in space vehicles; the detection, isolation and mechanisms of action of anticancer and antitumor antibiotics; a discussion of halucinogenic fungi and their potential uses by a neuropsychiatrist; the need for and the chemical properties of sanitizing agents in the textile industry; commercial application of deterioration of proteins with emphasis on tanning; deterioration of wood by ascomycetes and fungi imperfecti with a discussion of the necessary physical conditions, the species involved and standardization of laboratory tests or assay of activity; fungal metabolism and fungicides including material on metabolic changes induced in fungi by toxic agents as well as detoxification of chemical agents by fungi; types of variation in fungi and bacteria and the method by which a mutagenic agent exerts its effect. Aside from the aforementioned symposia, there are presented a few individual papers on such themes as use of griseofulvin in treatment of ringworm of mice, estimation of bacteria in oil field waters and the decomposition of cotton cellulose by Aspergilli. A final chapter in the book is devoted to the annual address of the president of the Society for Industrial Microbiology.

It must be obvious to the reader that there is a definite requirement for a book of this type issued on an annual basis.

There are only a few printing errors. The book is very readable and well presented. Although there is no index the organization of the material permits specific data to be located with ease.

HAROLD H. KUEHN

Bact. Res., Campbell Soup Co.
Camden, N.J.

GATÉ, J. & COUDERT, J.: Traitement de Mycoses. 279 pp. G. Doin et Cie. 8, Place de l'Odéon, Paris-VI, 1958. Price: NF 29.50.

Two outstanding authors of the Lyon School of Medicine present an interesting treatise on the therapy of mycoses, superficial and systemic. However, this work is not a list of drugs and procedures. Far from it. In the first part of the work they describe the general characteristics of the mycoses based on the fundamental principles regulating the biology of the fungi: saprophytism, their mode of aggression, including the general principles of treatment. In the second part, the authors present the systematic study of the divers syndromes of the mycoses, their etiology and pathogenesis, their essential symptomatology, the general rules of their treatment. The presentation of the methods of treatment is well balanced: beside the typically French therapeutic methods, those of the German and American Schools are fully considered. A thorough bibliography of 244 titles covering the French, German and particularly the American literature adds to the usefulness of the treatise. The authors can be highly recommended for their painstaking task to bring the extremely scattered therapeutic literature of mycoses under one cover. This work definitely fills a gap in the international mycological literature and it can be warmly recommended.

TIBOR BENEDEK

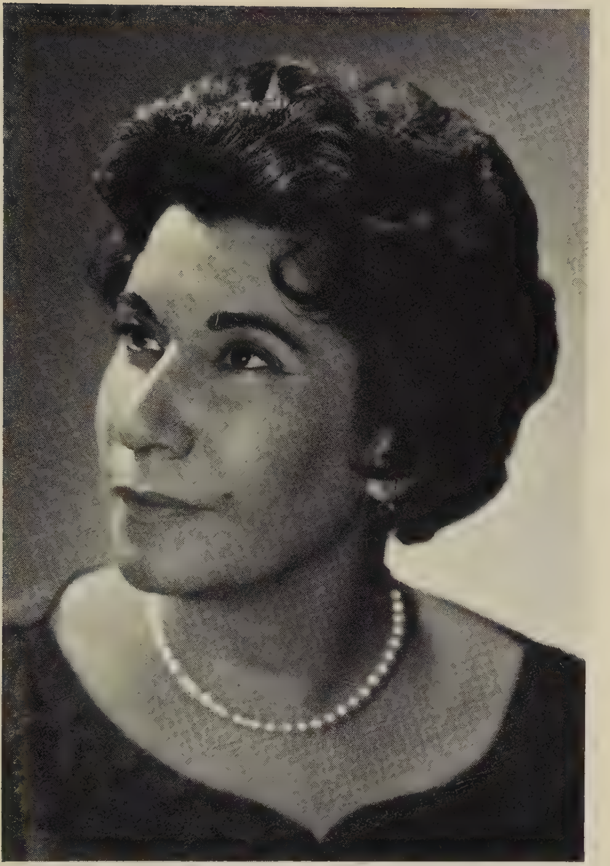
AGATHE VAN BEVERWIJK

Director, Centraalbureau voor Schimmelcultures
Baarn, The Netherlands

E. DROUHET

Service de Mycologie
Institute Pasteur
Paris, France

Gallery of Contemporary Noted Mycologists



MARIA DE LOS ANGELES SANDOVAL

Instituto de Salubridad y Enfermedades Tropicales
Departamento de Dermatología Tropical
México, D.F.

Gallery of Contemporary Noted Mycologists

S. SZATHMÁRY
University of Debrečen
Debrečen, Hungary

SOME NEW DISEASES OF HARDWOODS AND BAMBOO IN PAKISTAN

by

ABDUL HAMID KHAN

M. Sc. (Pb.) Ph. D. (Calif.)

(*Forest Botanist, Pakistan Forest Inst. Abbottabad, Pakistan.*)

(5.XII.1960)

(with 26 figs.)

Hardwoods are as important as any of our conifers. They are spread over hills and plains and constitute an important item in our economy. In this paper some of the diseases noted on our native hardwoods are presented.

DALBERGIA SISSOO DISEASES.

Shisham (*Dalbergia sissoo*) is an important forest species which constitutes the main species of forest Plantations. Although a valuable hardwood, it is attacked by a number of pathogens in the field. Besides minor diseases such as powdry mildew (*Phyllactinia corylea*) and the leaf rust (*Uredo sissoo*), it suffers from wilt due to *Fusarium oxysporum* and *Rhizoctonia solani* (BAGCHEE 1945, MAHMUD 1951). Root rot due to *Ganoderma lucidum* is a menace in the plantations. In addition to these a new root rot has been observed in some plantations which is described below:

Poria Root Rot.

This root rot was first noted in Van Bachran Forest Plantation in compt. No. 100, Mianwali district. Enumeration of dead and healthy trees was carried out and a map of the diseased areas was prepared (Fig. 1). The disease spread through 8 acres in two years. There was 61 % mortality of trees, varying from 4.6″ — 8.0″ diameter at d.b.h. It spread from a small patch to a large area in the same compartment. However, mulberry plants both young and about 5 years old were seen here and there which were free from the disease. Shisham trees 7 years old which had very little of heartwood suffered most from disease.

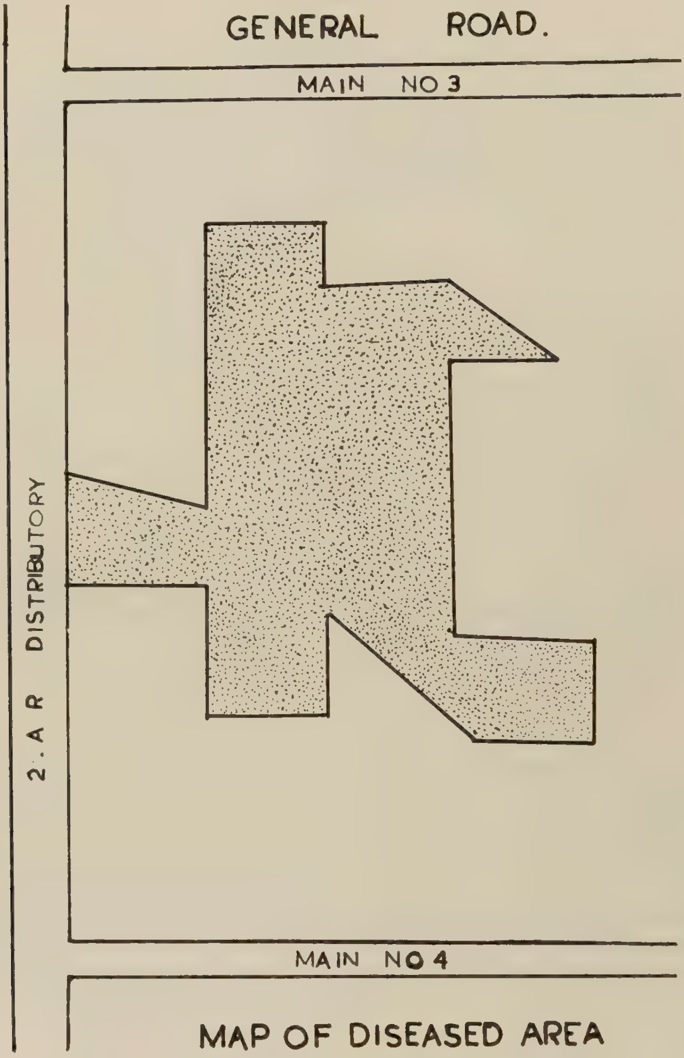

GENERAL ROAD.
MAIN NO 3
2. A R DISTRIBUTORY
MAIN NO 4
MAP OF DISEASED AREA

FIG. 1

Fig. 1

Symptoms.

The trees in early stages of infection were marked by thin, pale foliage, "Stagheadedness' and death of the tree. The fungus, at this time, formed a cheesy growth at the root crown and was visible at the base of the trunk (Fig. 2). The fructifications were formed at the base of the trunk where plenty of moisture was

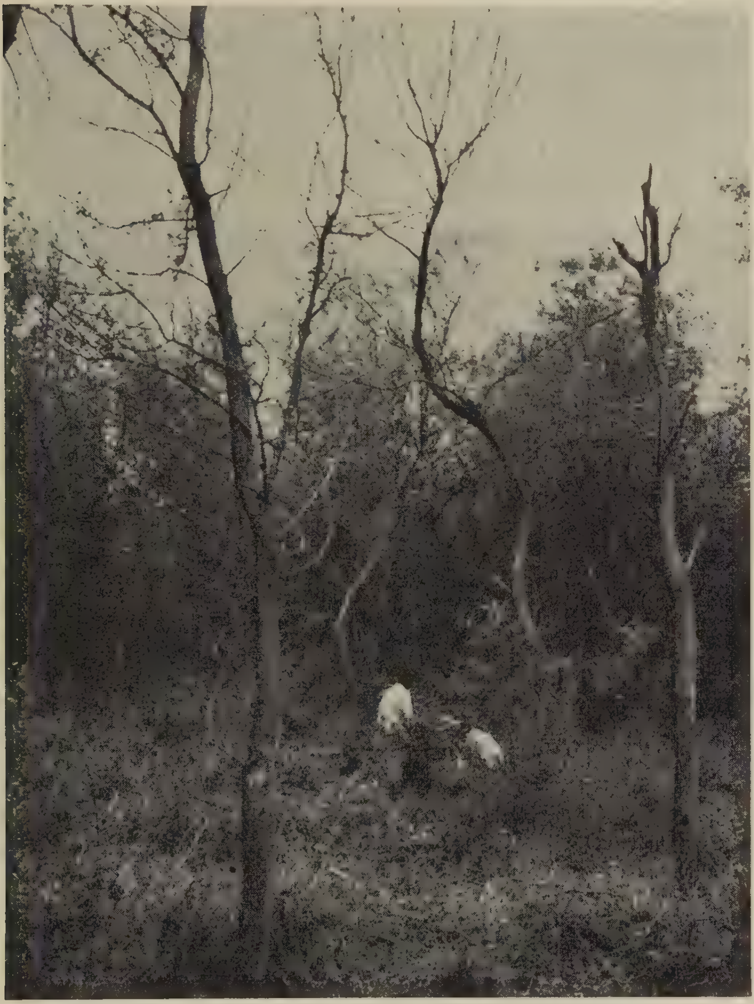


Fig. 2

available (Fig. 3). The root system was involved and dead trunks were riddled soon after, by *Sinoxylon* beetles.

The wood in the final stages becomes soft and spongy. The butt of standing dead trees becomes filled with hyphae upto a height of about two feet. Even the tunnels of *Sinoxylon* beetles get filled by with fungus growth. The wood becomes loose along the rings. The hyphae are white when fresh but later on the mycelium becomes buff to biscuit coloured.

Mode of Spread:

It has been seen that the trees in the Plantation were flood irrigated. Injuries at butt and collar portion of the stem due to digging of channels and cultivation etc. have apparently provided courts of infection. The fungus, it is estimated, kills the trees in about two years i.e. two summers usually elapse before the tree finally dies and is removed.

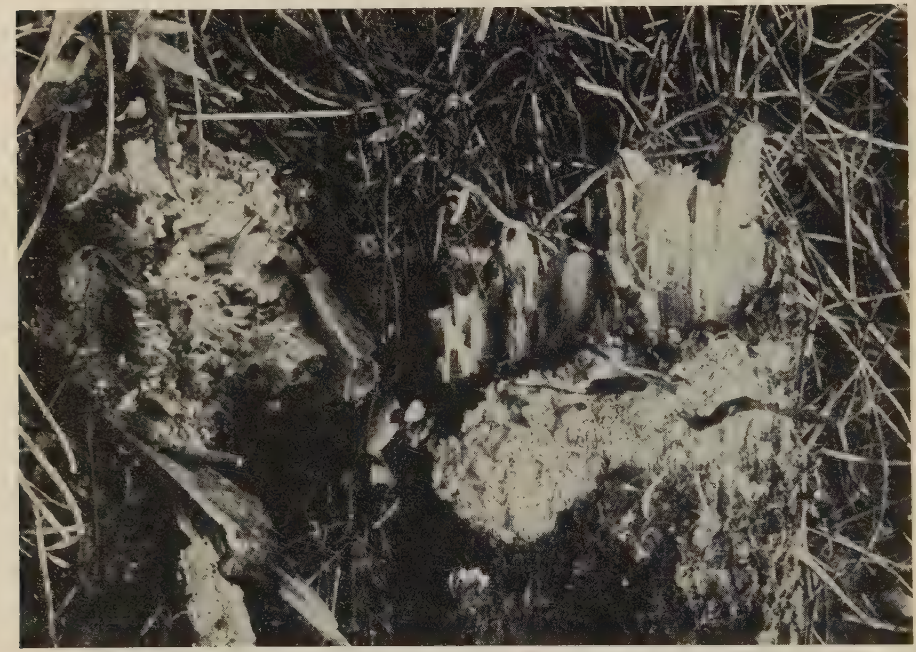


Fig. 3

It is clearly indicated that the disease spreads through (i) root contacts, (ii) carriage of mycelium or infected wood and (iii) spore dissemination from fruit bodies. Water as a medium of dispersal seems to be most important in this case. As will be apparent from Fig. 1, the mode of spread of the fungus is along the directions of water channels, and from one centre in all directions though in no way uniform. The roots were dug and majority of them was affected. The rotting wood was spongy. The fungus mycelium and fruit bodies were seen at the base (Fig. 3). The trees had died in patches as could be seen from the blanks in the plots of both the species (Fig. 2).

Causal Agency:

The fungus examined in these cases has been identified as *Poria* *ambigua* Bres. This is the first record of the fungus in West Pakistan on Shisham. It is a ubiquitous fungus, is facultative parasite and spreads rapidly under warm humid conditions as is evident from the foregoing description of diseased trees and sites.

Poria ambigua, Bres. Atti R. Acc. Sci. Lett. et. Arti, Agrati, 2. Ser. iii. fasc. 1—2. 1897.

It has a wide spreading sporophore with upper margin often inflated to sub-reflexed, white, finally straw coloured to Isabelline in colour. Subicle membranous, closely adnate, white. Tubes many, oblique to densely nodulose, mostly upto 8 mm long; pores somewhat larger, angular; finally dentate to lacerate (torn of) Spores are hyaline, obovate, 5—8 × 3—3.5 μ. Basidia clavate, 18—20 × 6—8 μ, cystidia fuscoid, apex muricellate (purplish & projecting) to finally yellowish 24—30 × 6—8 μ. Sub-hymenial hyphae delicate, tunicate, septate 3—4 μ broad. (Fig. 4).

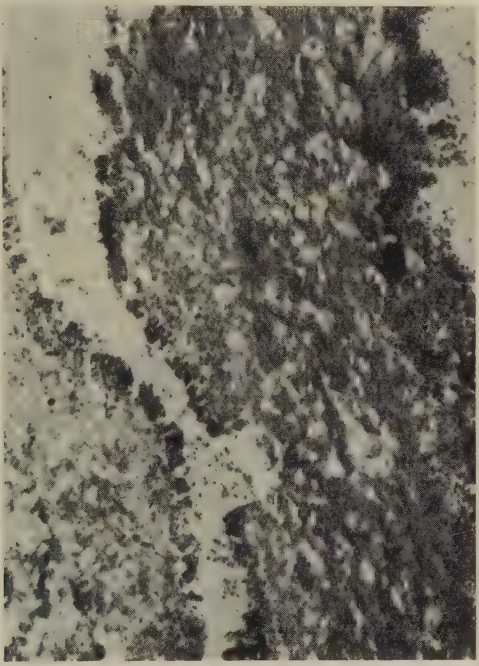


Fig. 4

This fungus has also been reported from Hungary on trunks of beech, *Carpinus, Pyrus* and *Prunus communis*. Also recorded on *Robinia pseudoacacia*.

Control Measures

Trenching and isolation are very difficult under flood irrigation. It is therefore necessary that the Shisham trees should be converted, stumps dug out and burnt on the spot. The irrigation channels should be blocked much before the approach of the diseased area and diverted, so that water from the infected site does not flow to healthy area. The debris should be collected and burnt on site of infected stumps. The area so cleared may be tried under cereal crops for 2 to 4 years before planting. A careful watch should be kept for the signs of fungus and its revival in the affected area on other plants.

2. Bombax Malabaricum (Salmalia malabarica) Diseases

Semul or Indian kapok tree is a quick growing soft type of hardwood which is much in demand for match industry and manufacture of packing cases. The young roots of this species are used in native medicine. The floss on the seeds is used as kapok. It is found in both wings of Pakistan. In East Pakistan it attains a much larger size and develops large buttresses. The present forest policy is to increase its plantation in West Pakistan. In the former Punjab it has been grown along the forest roads, in the Plantations.

Because of its quick growth, it suffers from frost and soft aerial parts of current year's growth are invariably killed in the former Punjab and farther north. But once the wood is mature and has developed thorns, it is not susceptible to cold. It extends successfully to Haripur and Havelian (Hazara District) where it has been planted along the roads and where frost is more severe than in the plains.

Fig. 5

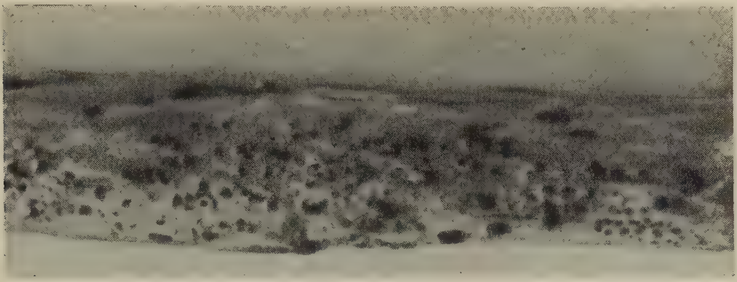


Fig. 6a

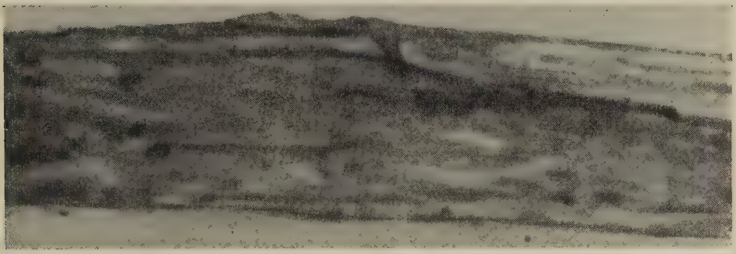


Fig. 6b

Pathogens recorded on *Bombax malabaricum* are *Diplodia Bombacina* on leaves; *Fusarium semitectum* (on seeds) and *Helminthosporium sativum* (on seeds), (AHMAD 1956), and semul also suffers heavily from *Loranthus* in East Pakistan (Fig. 5). Young plants also suffer from *Fusarium* wilt and attack of *Colletotrichum gloeosporioides.*

Phomopsis salmalica, KHAN, Spec. nov.

This fungus has been found generally, to attack young and succulent shoots. Young plants of 4 years or less are severely affected. The upper 4 ft. of the top are often killed. The bark gives a fermenting odour and is brownish and pulpy. The branches dry up and fruit bodies of the fungus burst out from the bark (Fig. 6 a,b). This die-back of the young fleshy shoots continues to weaken the plant till it dies. It is possible that twigs weakened at first by temporary drought or frost predispose the plant to fungus attack. But the death of shoots of plants growing along the canal bank during summer as noted in Pirawala indicate that the fungus is also an active parasite under warm and humid conditions.

Description of Pathogen

The fructifications are confined primarily to the bark, cambium and cortex. Small black pin points emerge out of the surface of the

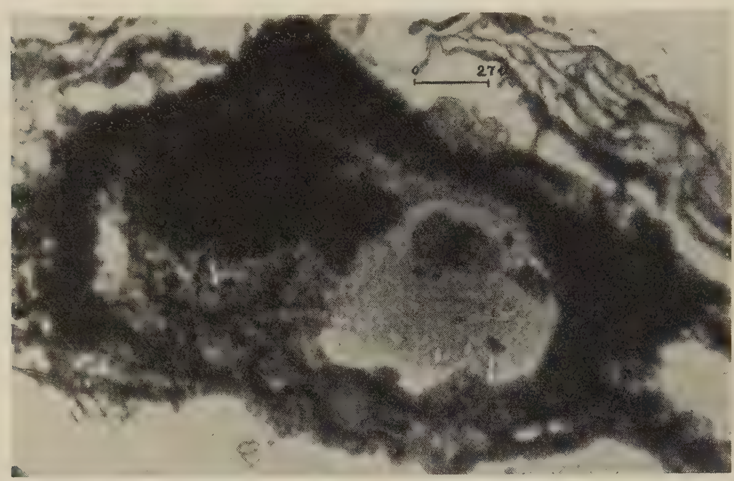


Fig. 7a

stem and branches (Fig. 7). The pycnidia measure 74—193 × 12—48 μ inside the cavity. Pycnidia are laterally and sometimes irregularly compressed, maculiform. The wall is 10—70 μ thick, outer layer thick celled and pseudoparenchymatous. Neck is short, 28—35 μ × 11—13 μ. Pore irregularly round conidiospores hyaline oblong, straight one celled, obtuse or indistinctly attenuate 4—7 μ × 1—3 μ. On dead stem and branches.

Fructificationes (Fructi) praecipue termenantur ad cortex. Puncta parva nigra ex facie trunci et ramorum erumpunt. Dimensiones pycnidiorum 74—193 μ × 12—48 μ. into vaco. Pycnidia a latere et interdum inaequaliter compressa sunt maculiformiter. Paries crassus est 10—70 μ, paries externus cellulas crassas habet et pseudoparenchymatosas. Cervix brevis est 28—35 μ × 11—13 μ: Formen rotundum in aequale est. Conidiosporae hyalinae, oblongae, erectae cum una cellula: hebetes aut (indistincte attenuatae) 4—7 μ × 1—3 μ. In trunco mortuo et ramis mortuis *Salmeliae* (*Bombacis Malabaricae*) Changa Manga et Chichawatni, Pakistan.

Control

Removal of predisposing causes is the only remedy feasible under forestry practice. The diseased twigs should be pruned off early in spring and burnt. The water relation of the plant should be adjusted in such a way that the tender shoots stop their growth sufficiently before the frosts set in.

AESCULUS INDICA DISEASES

Indian horse chestnut occurs in North Western Himalayas at 4.000—9.000 ft., in Pakistan. The tree is met with in moist locali-

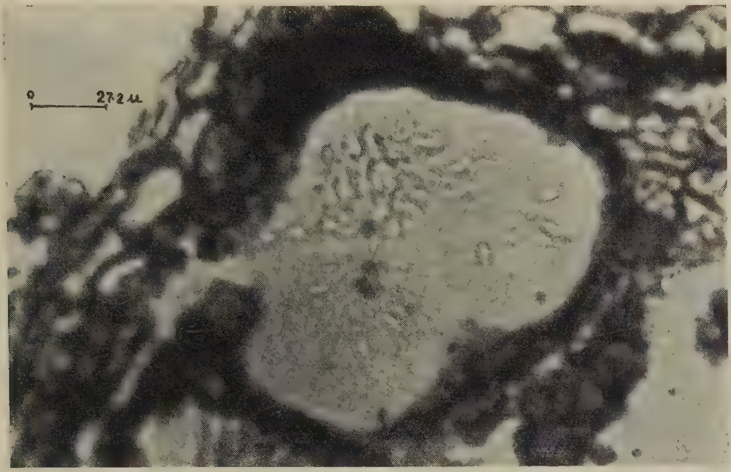


Fig. 7b

ties and is gregarious in some places. The wood is pinkish or reddish, soft and close grained. It is used for turnery goods and mathematical instruments. The leaves are used for fodder. It suffers from a die-back in Muzaffarabad (Azad Kashmir) area.

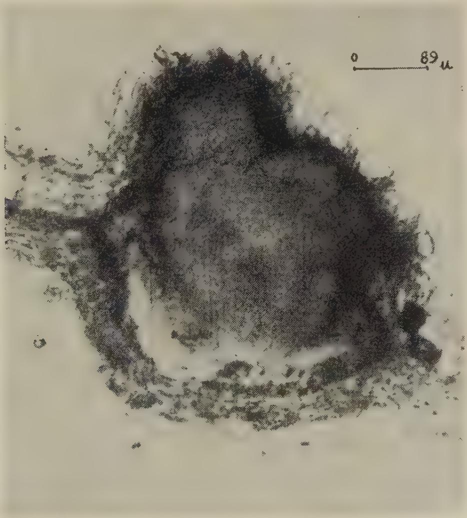


Fig. 7c

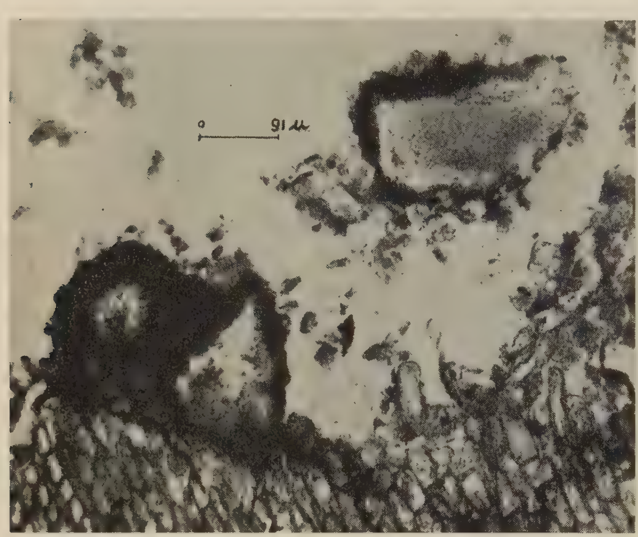


Fig. 7d

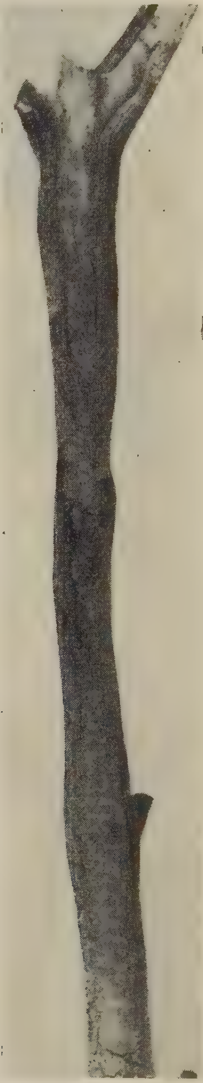


Fig. 8

Die-back disease.

The affected trees show die-back of branches. The bark dies, exfoliates and drops bit by bit. Canker is formed and wood is exposed (Fig. 8). The branches may be affected simultaneously so that the tree looks sickly (Fig. 9). In final stages stagheadedness is visible. The tree suffers in growth and declines in vigour and is later affected by insect and physical agencies.

The disease is more common on trees situated on the banks of river on bouldry sites.

Causal Agency.

Botryosphaeria ribes. G. & DUGG is the causal fungus. It is also the cause of blight of canes of currants. *Botryosphaeria ribes* G. & DUGG. has black stroma which is more or less pulvinate, outer

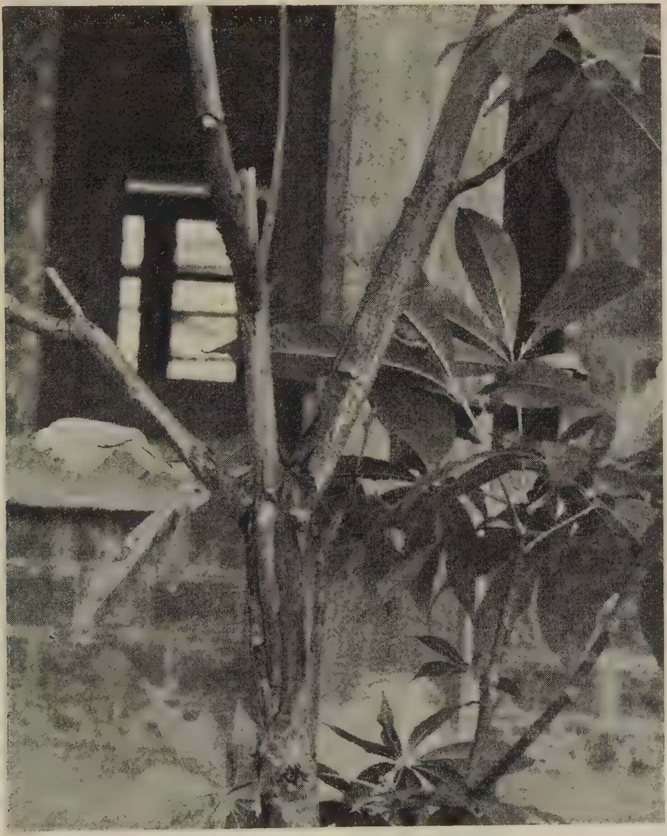


Fig. 9

surface is botryose, 1—4 mm in diameter, usually 2—3 mm: and surrounded by the fissured periderm, regularly scattered or in more or less definite longitudinal rows or elongated stromata. Perithecia are somewhat top shaped, with papillate ostioles and usually projecting; sometimes practically superficial, few to many in stroma and usually interspersed among pycnidia, 175—250 μ, in width. Asci clavate, 80—120 × 17—20 μ, and with numerous filiform paraphyses. Spores fusoid, hyaline, continuous, 16—25 × 5—7 μ, (Figs. 10a, b).

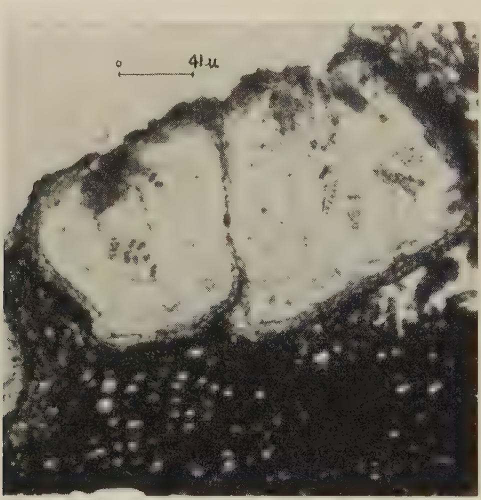


Fig. 10a

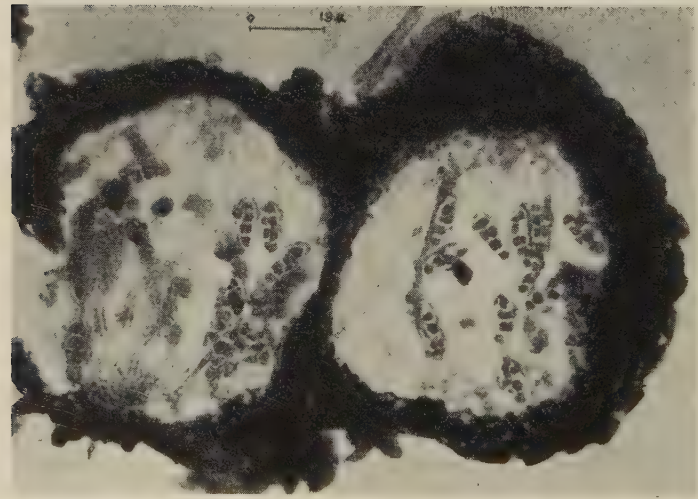


Fig. 10b

Pycnidia of the compound stylosporic form — *Dothiorella* are borne in the same or similar stromata; spores fusoid, continuous, hyaline, 18—31 × 4.5—7 μ. Pycnidia of the simple stylosporic form—*Marcophoma*, are embedded in the outer bark under the much raised primary cortex or young shoots, depressed, globular, 175—250 μ wide; spores fusoid, hyaline, continuous 16—23 × 4.5 μ.

4. PRUNUS CORNUTA (PRUNUS PADUS) DISEASES.

Kalakath or Bird cherry, is moderately durable wood, used in sports industry, rifle butts and locally for turnery goods. It is worked for firewood as it regenerates well from root suckers.

Leaf Spot

This tree suffers from a leaf spot. The disease is found in moist sites and closed situations along the north and western slopes. The leaf spot is conspicuous during summer and becomes worst after rains. The leaves first show light green or yellow spots which later develop reddish yellow swollen areas. Later on during the rains the spots become more numerous and almost 60—70 % of the leaf area may be covered by the reddish or orange areas which later turn brown to fuscous (Fig. 11). By the time the fruit bodies ma-

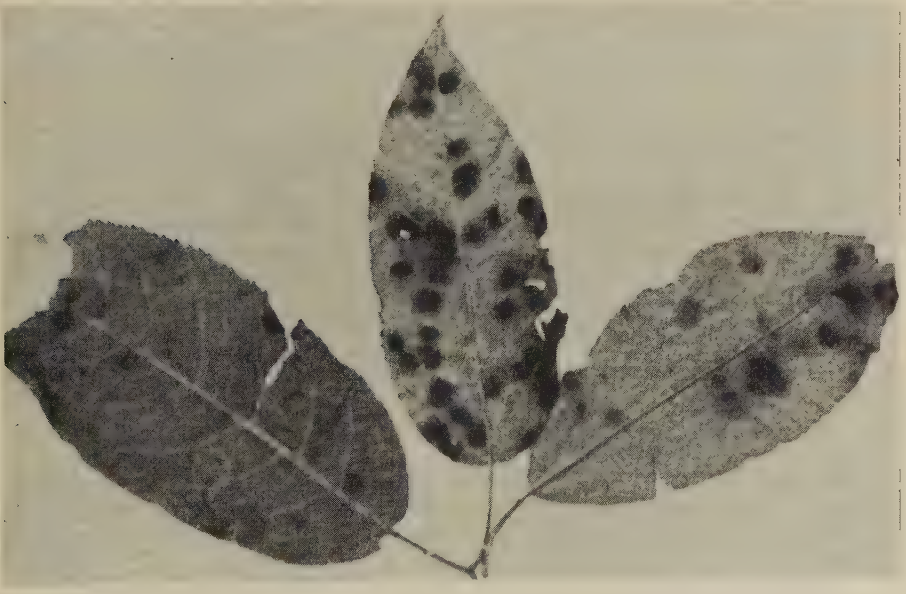


Fig. 11

ture, the affected leaves fall to the ground prematurely. The maximum infection is seen early in september and the tree presents a parched orange brown appearance while the healthy trees with slight attack are still green. Because of early defoliation and reduction in photosynthetic activity there is a reduction in vigour and growth of trees. On the northern slopes and in depressions the trees are mostly affected. It is noted that when the infection reaches its maximum the plant is ready to enter the dormant period and during the last two months the growth is not vigorous. Hence the effect on the trees is not very marked. Re-infection next year occurs most probably through ascospores on dead leaves and further multiplication occurs through conidial stage.

Causal Organism.

The causal organism is *Polystigma ochraceum* which closely resembles *P. rubra* and is easily confused with the former even by the expert. Salient features are detailed in the succeeding paragraphs.

Polystigma ochraceum (Wahlenb.) Sacc.
Polystigma fulvum Dc (1817);
—*Sphaeria ochracea*), has been reported on living leaves of *Prunus cornuta* (*P. padus*) and *Prunus cerasi* from Finland, Russia, Britain, Italy, Germany, France, Belgium and Switzerland. The variety *aurantiacum* West is found on leaves of *Crataegus* and *Pyrus* in Holland, France and Germany. This species resembles closely *Polystigma rubrum* from which it differs in size of asci and spores. The descriptions (Saccardo 1931 & Petrak 1954) are given in the table below:-

Polystigma ochraceum (Wahlenb.) Sacc.	*Polystigma rubrum* (Pers.) Dc.
(= *Sphaeria ochraceum* Wahlenb. (Lapp) (= *Polystigma fulvum* D. C.) Stroma on both surfaces of leaves subangular, spreading 5—10 mm wide, when fully formed mostly confluent. Ochraceous to fulvus, white inside. Loculi immersed. Ostiole impressed but conspicuous when on the lower surface.	(= *Xyloma rubrum* Pers. = *Dothidia rubra*) Stroma hypophyllus, suborbicular. Reddish to brown. Loculi immersed. Ostiole impressed.
Asci and spores as in *P. rubra.* but a little larger. Asci 70—80 × 13—15 μ. Spores 14—15 × 6μ.	Asci clavate, 8-spored, spores ovoid, obtuse, straight subhyaline. 10 × 6 μ.
Imperfect Stage.	Imperfect Stage.
=*Libertella fulva* Thum.	=*Libertella rubra* (Desm.) Bon.

=*Polystigmina pallescens* PETR. 1954. Sydowia, iii
Stromata amphigenous, orbicular to elliptical, 2.6 mm diam. Pallid ochraceous or dark flavescent, fleshy

Pycnidia 300-450 μ. in diam. immersed in stroma, depressed, globose to ellipsoid. Ostiole 20 μ across, pappiliform, punctiform, variously curved, continuous, 30—50 μ rarely 60×1μ.

= *Septoria rubra* DESM. = *Polystigmina rubra* (DESM.) SACC.
Stromata hypophyllous, suborbicular fleshy, flat-convex; red later rufo-fuscous.

Pycnidia numerous, ostiole punctiform, subpolystichous. Spores hyaline, filiform, straight subuncinate, 6—9 nucleate 25—30 × 1.5 μ.

Since *Polystigma ochraceum* (WAHLENB.) SACC. has already been described and occurs in Pakistan on *Prunus cornuta* (=*Prunus padus*) the imperfect stage described as *Polystigmina pallescens* PTR. most probably belongs to *Polystigma ochraceum* for which an imperfect stage *Libertella fulva* THUM. already exists; therefore, the recently described species may be taken as synonym of the imperfect stage of *P. ochraceum* (WAHLENB.) SACC. as shown above.

Control Measures.

There is little doubt that the disease reduces the photosynthetic activity and deprives the tree of its much needed food. The disease is at its maximum when the tree is entering dormant stage, therefore, the intensity of damage is reduced. No special measures are ordinarily necessary unless there is an epiphytotic. In such a case, removal of congestion and encouraging mixture of species will be quite effective. Destruction of leaf litter.

ALBIZZIA ODORATISSIMA DISEASES.

It is a large almost evergreen tree, found in both East and West Pakistan. *Albizzia* gives moderately durable wood which is dark brown with darker streaks. It is subject to end and surface cracking during drying, otherwise is a fine timber which takes good polish after proper finishing. In Chittagong Division (Baryadhala) it is severely attacked by a leaf spot disease.

Leaf Spot.

The leaves are attacked mostly in spring or during the rains. The affected leaves at first show yellow pin points. These slowly produce black points which protrude and burst open (Fig. 12). The affected leaves keep on hanging and every affected tree had almost 80 percent leaves spotted. The reduction in the leaf surface for photosynthesis is very significant although in old trees adverse effects were not manifest. The leaves on the top portion of crown were most affected. The infection on leaves at the lower part was very little. The fruit bodies showed spore tendrils coming out of their ostioles. The fogs and mists in the area help in the germination of spores and their dissemination. The disease is more in wet spells.

The causal organism has been identified as *Endodothella albizziae* (SYDOW) THEISS.

Description of Pathogen.

Endodothella albizziae has epiphyllous stromata, which are sparse, round, minute, 0.2—0.5 mm diam. black inside, mostly unilocular rarely bi-locular. Asci clavate, apex round 55—75 × 14—19 μ; 8-spored. Spores obliquely monostichous or distichous, ellipsoidal, finally round 1-septate in the middle and very much constricted; at first hyaline, finally slightly dark, 12—16 × 5—6 μ.

It has also been observed on the leaves of *Albizzia marginata* from Philippines.

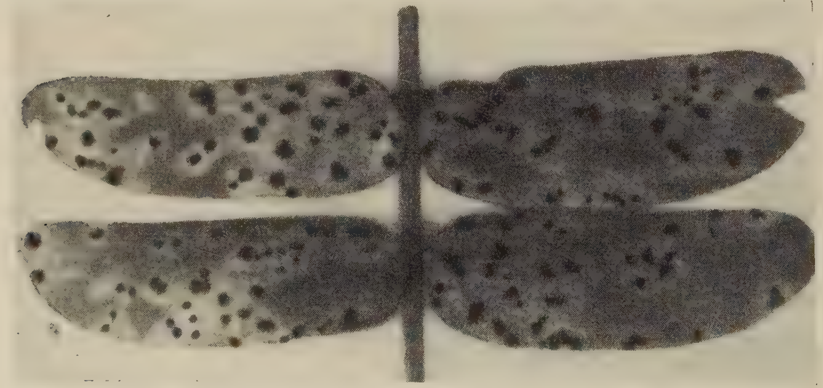


Fig. 12

POPULUS NIGRA DISEASES

This is a common tree grown in Kaghan, Hazara District and Peshawar Valley, from 2,000—10,000 ft. It is a handsome avenue tree which grows easily from cuttings. It does not do well in the plains. It suffers from a severe die-back in some localities (Fig. 13).

Die-back diseases

The disease gradually kills the branches. The tree loses its young shoots to begin with and ultimately cankers are observed on the main limbs which may in turn be girdled and dry up. The tree looks stagheaded and young shoots are given off from the basal living

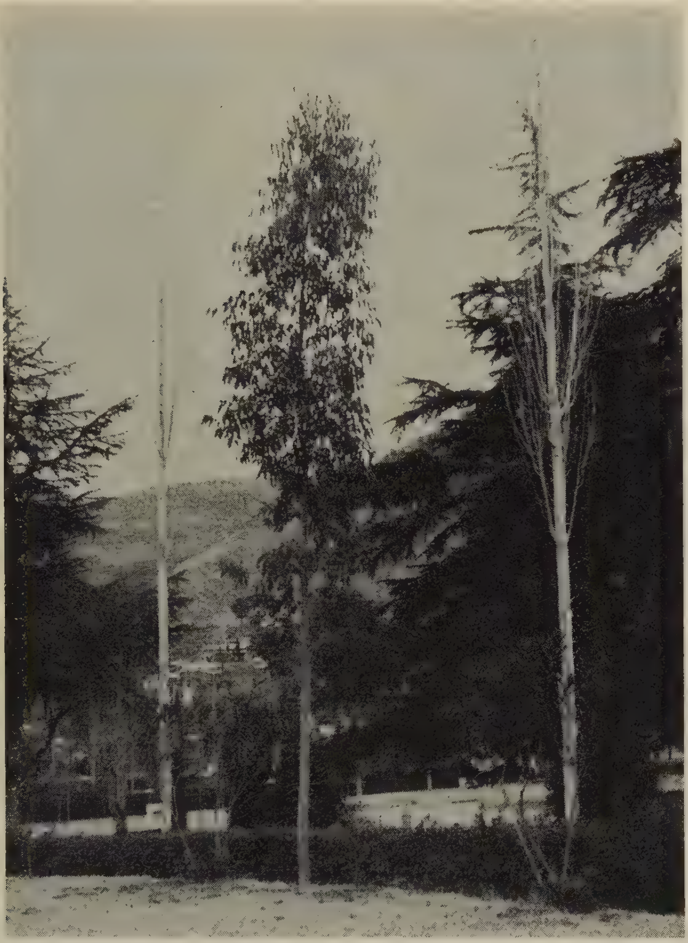


Fig. 13

stem becoming crowded at some points. Many of the young shoots are killed and new ones continue to grow successively, decreasing in vigour.

The causal fungus is *Botryosphaeria ribes* (Fig. 14). Along with this, *Lophiotrema nucula* has also been noted. The description of *Botryosphaeria* has already been given under diseases of *Aesculus indica.*

Pruning of diseased and dead twigs much below the dead wood and dressing up or cuts with antiseptic paint are advocated.

Recently *Ganoderma lucidum* has been detected to cause root rot in hybrid clones of *P. euramericana* being grown in Lahore by the Silviculturist. It has come from Shisham trees which are growing there.

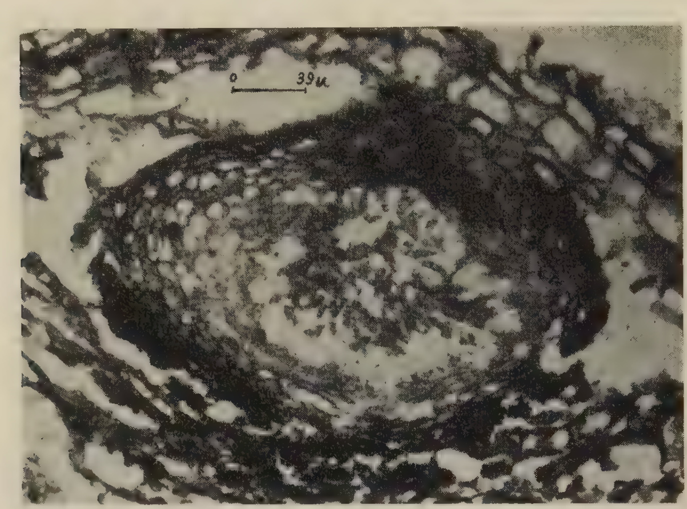


Fig. 14

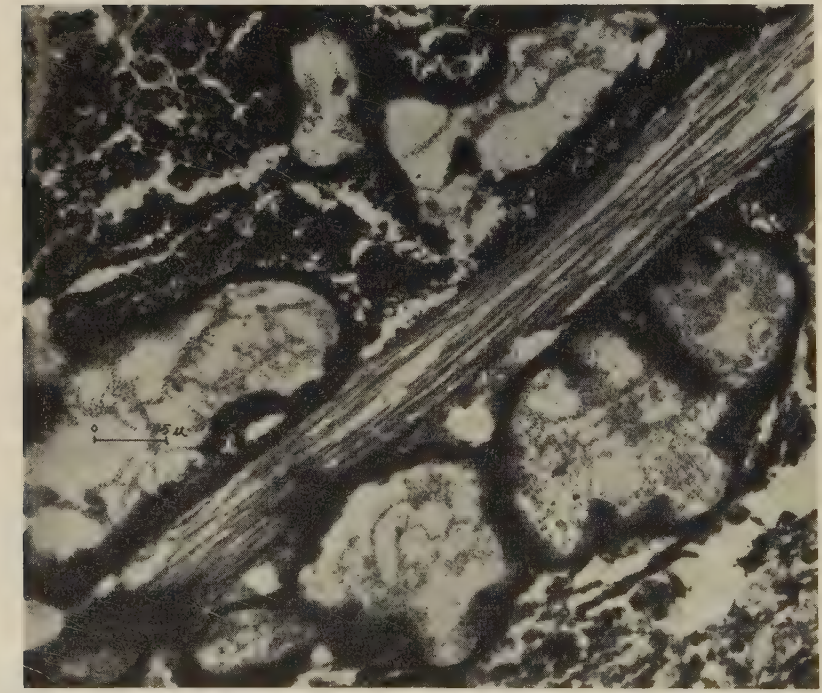


Fig. 15a

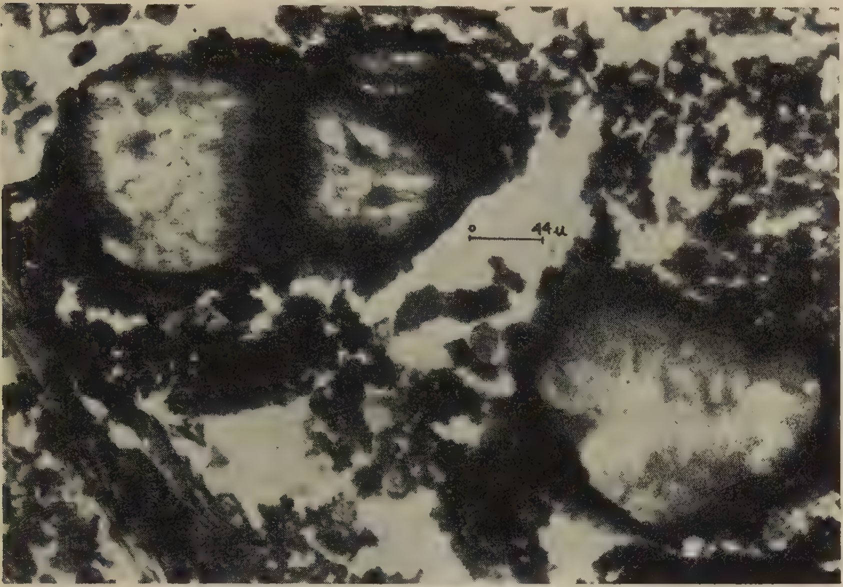


Fig. 15b

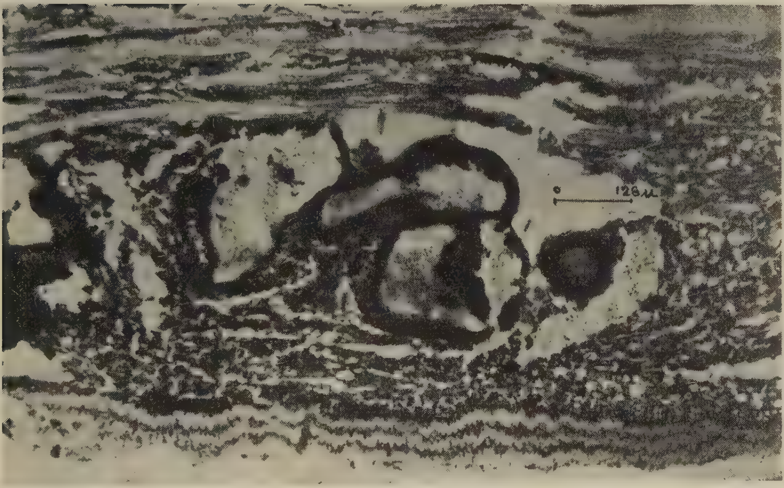


Fig. 15c

SALIX DENTICULATA VAR. HAZARICA, DISEASE.

It is a common shrub of the forests, shoots being used for basket making. The new sprouts are killed and growth is stunted. On killed shoots fruit bodies of *Valsa dolsa* have been collected. The fungus is seen in moist localities at 6—8.000 ft.

This is a wide spread fungus found in various parts of the globe. The Asci are narrow, oblong, sessile, 8-spored, $64 \times 10 \mu$; spores subdistichous, cylindrical, curved, hyaline inside; spermatia allantoid $6—7 \times 1.5\ \mu$ long pedicellate. (Fig. 15, a—c)

This fungus is also found on branches of *Salix caprea,* and *Celastrus* sp.

BAMBOO CULM SPOT

Melocanna Bambusoides culms are generally affected in East Pakistan. Large number of spots are present on the culms. Though very little damage is done so far as the utility of the bamboo culm is concerned.

But where appearance is of prime importance, the disease certainly causes degrade. The causal organism is *Apiospora Montagnei* (Fig. 16).

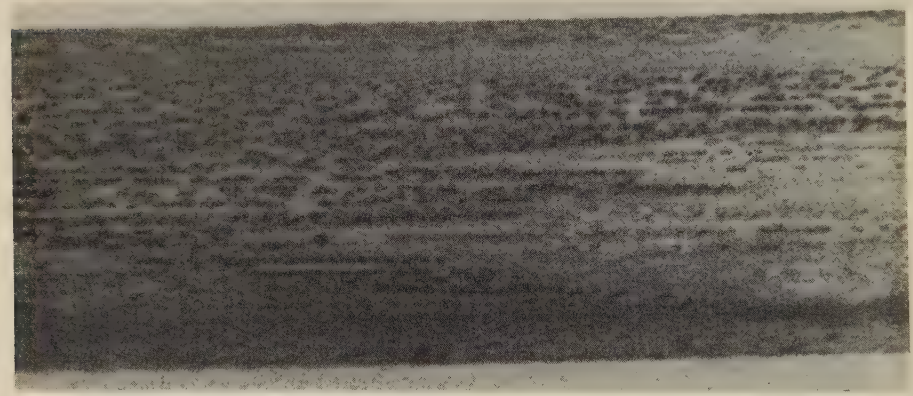


Fig. 16

Apiospora indica THEISS and SYD. (Ann. Mycol. 13, 1915) has been reported on leaves of Bambusae from India (Wynaad) by BUTLER. *Apiospora carbonacea* is a closely allied species reported on *Schizostachyum.* It differs from *Apiospora indica* especially in perithecia being more carbonous and receding.

Apiospora Montagnei SACC.

Stroma erumpant, linear, black. Perithecia comparatively large,

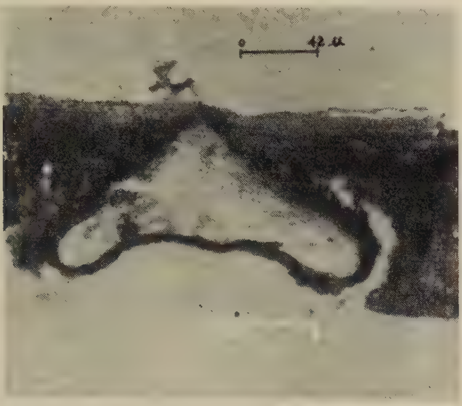


Fig. 17a

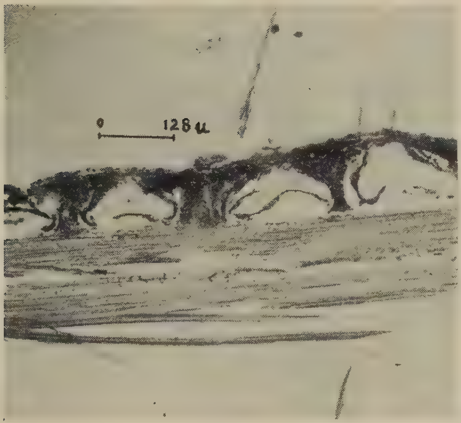


Fig. 17b

globose, in one row, completely full, stromate, fuscous, united; ostiole hemisphaerical umbilicate (Fig. 17 a,b). Asci clavate, 70—90 × 20 µ, Spores biseriate, ovate-oblong, appendiculate to pyriform 25—2 × 10 µ, hyaline.

It has also been reported on culms of *Arundinaria mauritanica* from French Perpignan and Algeria, as well as from Carobra and Alabama in America. This species has also been recorded on *Bambusae* in Nangki in India.

Acknowledgements

The author is grateful to authorities of Commonwealth Mycological Institute, N.Y. Botanical Garden and Dr. S. AHMAD, Government College, Lahore, for their help in the identification of the material sent and supply of scientific material for study.

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EFFECT OF COTTON DUST AND 2,4-D ON HOST-PARASITE RELATIONSHIP. (I) COTTON AND FUSARIUM-WILT.

by

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I. INTRODUCTION

Cotton dust has been extensively used in Egypt in the last few years for combating the cotton leaf worm. Cotton dust is a mixture of these insecticides: sulphur, dichlorodiphenyltrichloroethane (DDT) and the gamma isomer of benzene hexachloride (BHC). As the cotton dust components DDT and BHC are known to be persistent in the soil (Smith, 1948 and Fleming, 1950), different concentrations of cotton dust — representing different degrees of the insecticide accumulation in the soil — were dealt with. In addition, a comparative study has been carried out to test the herbicide 2,4-Dichlorophenoxyacetic acid (2,4-D) on the host-parasite balance of cotton-wilt disease due to *Fusarium solani*.

Many investigators recorded that DDT has no effect on the host-parasite relationship (Heuberger & Strearns, 1946, Macdonald, 1948, Davidson & Rich, 1947 and Sackston, 1949). On the other hand, Johnson (1946) found that spraying wheat plants with DDT — four days after being infected with *Puccinia graminis tritici* — resulted in the formation of larger uredosori than those formed on control plants. DDT was recorded by Bowser (1946) to have an indirect effect on the potatoes against aster-yellow virus disease through inhibiting the carrier "leaf hoppers". Bonde & Snyder (1946) recorded that potato plants sprayed with basic copper sulphate, to which DDT or BHC was added, caused good control of early blight due to *Alternaria solani* and late blight caused by *Phytophthora infestans*. The control of early blight — due to DDT application — was attributed to delayed plant maturity and to the greatly reduced number of flea-beetle punctures, which afforded entry to the fungal pathogen. BHC was proved also to be effective in controlling the wheat bunt disease caused by *Tilletia caries* and *Tilletia foetida* (YERSIN and co-workers, 1947). Its gamma isomer was found to control the damping off disease of *Pinus* seedlings. Its application stimulated further mycorrhizal development in these seedlings. Concerning sulphur, FIKRY (1936 and 1948) found that sulphur dusting was effective in controlling the powdery mildew of cucurbits in Egypt - due to *Erysiphe cichoracearum* - and had a remarkable effect in increasing the yield of vegetable marrow plant. Similarly, it was found to be effective in controlling bean rust caused by *Uromyces fabae* (ZAUMEYER, 1946) and vine powdery mildew due to *Uncinula necator* (BIOXO, 1947). Sulphur was applied by PERSON (1946) to eliminate losses of sweet potatoes caused by soil rotting due to *Actinomyces ipomoea*; the action of sulphur was to shift the soil pH from a value favourable for infection (i.e. 5.6-5.8) to an unfavourable value (i.e. 5.0).

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2,4-D on the other hand was found to affect the host-parasite relationship in mould diseases. Thus, treatment of tomato fruits with 2,4-D caused a reduction in the number of fruit cracking due to fruit cracking moulds (SHROADER & SMITH, 1946). Similarly, GUISCAFRE-ARRILLAGA (1949) reported the inhibitory effect of 2,4-D to post harvest decay of orange due to *Penicillium digitatum, Penicillium citri* and *Phomopsis citri*. IBRAHIM (1951) found that spraying of oat plants with dilute solutions of 2,4-D butyl ester induced their resistence against *Puccinia graminis avenae.*

HSIA & CHRISTENSEN (1951) reported that 2,4-D treated wheat plants were weakened, stunted and predisposed to heavier infection.

CROWDY & WAIN (1951) recorded a reduction in the chocolate-spot disease of bean plants when dipped or sprayed with 2,4,6 trichlorophenoxyacetic acid. LONGCHAMP, ROY & GAUTHERET (1952) recorded the development of *Claviceps purpurea* in wheat fields due to their herbicidal spray with 2,4-D ethyl ester.

II. MATERIAL AND METHOD

Agrocide cotton dust, pure sodium salt of 2,4-D as well as Fernoxone (80 % 2,4-D, commercial) were kindly supplied by the Imperial Chemical Industries. *Fusarium solani* was isolated from wilted cotton plants. "Giza 26" and "Menoufi" cotton seeds were kindly supplied by the Plant Breeding Section, Ministry of Agriculture.

Sandy soil of the following mechanical analysis: Clay 1 %, silt 2 %, fine sand 70 % and coarse sand 27 % ,was prepared and steam sterilized. The sterilized soil was subsequently left exposed — for one week — in the laboratory to be aerated.

Soil treatment with cotton dust: Sterilized soil was then divided into four lots, three of which were mixed with the following concentrations of cotton dust: 10, 100 and 1000 p.p.m.; the fourth was left untreated to serve as control. The calculated amount of cotton dust per 10 kg of the soil was mixed in a mortar with 100 g of the soil. This in turn was mixed and shaken well to make up to one kilogram. The resulting kilogram of soil — treated with cotton dust — was then gradually distributed among the remaining nine kilograms. These successive stages of mixing ensure the thorough distribution of cotton dust in the experimental soil.

Soil inoculation: *Fusarium solani* was grown on Dox' liquid in one-litre flasks incubated at 25° C; a temperature which proved to be near the optimal. After ten-days incubation, the mycelial mats were filtered off, each was transferred into a litre of sterilized water, they were splitted into fragments and distributed evenly in the aqueous suspension by shaking in a blender.

Pots were surface sterilized, each received 250 cc of the mycelial suspension and mixed thoroughly with the cotton-dust treated or untreated soil. The inoculated pots were covered with sterilized paper sheets and left at ordinary temperature (i.e. 18°—28° C) for one week, during which they were irrigated regularly — every two days — with equal suitable amounts of sterilized water. At the end of this period, the fungus was found to contaminate the soil vigorously, and the pots were now ready for seed sowing.

Seed sterilization: Healthy seeds of the two experimental cotton varieties — namely, the *Fusarium*-wilt resistant "Menoufi" variety and the susceptibel "Giza 26" variety — were firstly delinted by soaking in concentrated sulphuric acid for ten minutes; this was proved helpful in accelerating seed germination through softening the seed coats. The treated seeds were subsequently washed thoroughly to remove all traces of the acid and were then transferred for sterilization, into 1 : 1000 mercuric chloride solution for two minutes. The treated seeds were then washed in a series of sterilized water to free them from the corrosive sublimate, before finally be sown in pots.

Accordingly, the pots were divided into the following four categories of soil treatments:

a. Sterilized uninoculated cotton-dust untreated soil.
b. Inoculated, 10 p.p.m. cotton-dust treated soil.
c. Inoculated, 100 p.p.m. cotton-dust treated soil.
d. Inoculated, 1000 p.p.m. cotton-dust treated soil.

Each category, consisting of eight pots, was further subdivided into two lots — four pots each — one lot was sown with "Giza 26" cotton seeds and the other with those of "Menoufi"; each pot received twelve seeds. The sown pots were placed in a glass house and examined daily for eight weeks. During this period, the pots were regularly irrigated with suitable equal amounts of sterilized water.

Treatment with 2,4-D: Germination of cotton seeds was found to be very sensitive to low concentrations of 2,4-D. On the other hand, *Fusarium* is much more resistant. Hence, the study of host-parasite relationship, in presence or absence of the herbicide, was approached indirectly by treating the pathogenic fungus itself before soil inoculation; this was carried out by a modified technique adopted by HSIA & CHRISTENSEN (1951). Thus, *Fusarium* was grown on Dox' liquid or on the same medium containing 200 p.p.m. 2,4-D, for ten days at 25° C; such concentration was found not to interfere considerably with fungal growth and viability. The treated mycelial mats were divided into two lots: one was thoroughly washed with distilled sterilized water and the other left unwashed. Accordingly, the three following treatments were made for *Fusarium* mats: a. Untreated, b. 2,4-D treated and washed and c. 2,4-D treated and unwashed. Soil inoculation was carried out in the same method previously described.

III. EXPERIMENTAL RESULTS

(1) Effect of cotton dust

The external symptoms of *Fusarium*-wilt disease of cotton manifest themselves as yellowing or mottling of seedling leaves (FAHMY, 1928) known as mosaic symptoms. FIKRY (1932) considered, however, that a true indication of the disease is the discoloration of the root fibrovascular tissues due to the presence of an associated gum with the invading pathogen; such discoloration takes the form of characteristic brown streaks when infected roots are cut longitudinally. Both criteria of infection were taken into consideration in the present work. The number of raised seedlings showing mosaic leaves were determined during a period of eight weeks from time of sowing; seedlings were then carefully uprooted, the roots washed and they were cut longitudinally to test for the presence of discoloration. Hence, the following criteria were determined for each particular type of soil treatment:

a. Number and percentage number of raised seedlings in comparison with sown seeds.

b. Number and percentage number of infected seedlings — manifested as mosaic and discoloration — in comparison with the number of raised seedlings.

Since yellowing or discoloration might be due to the mere presence of cotton dust in the soil, fungal isolates were made from the discoloured streaks and the identity of the causal pathogen was determined in every case.

The host-parasite relationship, concerning the experimental cotton varieties on the one hand and *Fusarium* on the other hand, does not seem to be appreciably disturbed in the presence of varying concentrations of cotton dust up to 100 p.p.m.; "Giza 26" cotton continues to be highly susceptible and "Menoufi" resistant to *Fusarium* infection. At 100 p.p.m. cotton dust, however, the percentage infected seedlings showing mosaic is considerably greater than those at 10 p.p.m. or untreated soil (Table I).

TABLE I.

Responses of either "Giza 26" (G) or "Menoufi" (M) cotton variety to Fusarium infection, as influenced by varying concentrations of cotton dust. The number of sown seeds is 48. The number (No.) and percentage number (%) of raised and infected seedlings is indicated.

Cotton dust concentration (p.p.m.)	Cotton variety	Raised seedlings		Infected seedlings showing:			
				Mosaic		Discoloration	
		No.	%	No.	%	No.	%
0	(G)	40	83	30	75	37	92
	(M)	42	87	3	8	5	12
10	(G)	38	79	26	67	32	48
	(M)	45	93	3	6	6	13
100	(G)	40	83	22	55	36	90
	(M)	41	84	8	19	1	2

An experimental difficulty was confronted with regard to cotton seedlings grown at the highest experimental cotton dust concentration (i.e. 1000 p.p.m.); discoloration due to infection was masked since roots of cotton plants grown in uninoculated soil became dark in response to such high concentration of the insecticide. Hence, fungal isolation has been carried out from the roots — as the only accessible infection criterion — to determine the host-parasite balance at such high concentrations of cotton dust. In all raised seedlings, of both susceptible "Giza 26" and the resistant "Menoufi" cotton varieties — the fungus was found to grow vigorously out of the tested root fragments; this denotes that at 1000 p.p.m. cotton dust concentration, *Fusarium* could penetrate the root tissues of the resistant as the susceptible cotton varieties. The penetration of *Fusarium* into cotton seedlings could not be considered, however, as an absolute criterion for infection as has already been proved by FAHMY (1928) and NAIM (1943). The latter author has demonstrated that in *Fusarium*-resistant cotton variety "Karnak" the fungus advanced into the root cortical cells, then it ceases to penetrate further and fails to establish infection; this was found to be due to the formation of a corky barrier in the pericycle which interfered with the further development of the fungus into the vascular tissue.

(2) Effect of 2,4-D

The results (TableII) showing the effect of 2,4-D on *Fusarium* wilt could be expressed as follows: The percentage raised seedlings,

TABLE II.

Number and percentage number of raised and infected seedlings of either "Giza 26" or "Menoufi" (M) cotton variety in response to Fusarium as influenced by different 2,4-D treatments; the number of sown seeds is 48.

Fungal treatment	Cotton variety	Raised seedlings		Infected seedlings	
		Number	Percentage	Number	Percentage
Normal	(G)	41	84 %	38	92 %
	(M)	45	93 %	3	6 %
2,4-D (washed)	(G)	39	81 %	32	82 %
	(M)	41	84 %	0	0 %
2,4-D (unwashed)	(G)	33	69 %	28	84 %
	(M)	36	75 %	0	0 %

on soil inoculated with 2,4-D-treated unwashed mycelial mats were found on the whole to be comparatively less than those on soil inoculated with either normal or 2,4-D-treated washed mycelial mats; this holds true for both cotton varieties. The low percentage infection of "Menoufi" seedlings by normal mycelial mats, was completely eliminated by the treatment of the mats, before inoculation, with 2,4-D, whether washed or unwashed. Similarly, the percentage infection of "Giza 26" cotton seedlings was comparatively less on soil infected with 2,4-D-treated *Fusarium* mycelium than in response to normal mycelium.

(3) **Effect of cotton dust and 2,4-D on the wilt-inducing potentiality of Fusarium metabolites.**

Previous work done on the cause of *Fusarium*-wilt of cotton has demonstrated the presence of a fungal metabolite or excretion responsible for wilting (FIKRY, 1932; MOSTAFA & GAYED, 1953; and NAIM, 1953). No work has been done to find out if treatment of cotton with agricultural chemicals would affect its response to the toxic action of *Fusarium* metabolites. Accordingly, the present experiments are an attempt to elucidate the following points:

(a) The relative wilt-inducing capacity of *Fusarium* metabolites to cut shoots of each cotton variety.

(b) Comparisons of the wilt-inducing capacity of the fungal metabolites to cotton cut shoots, when the latter was previously sprayed with either cotton dust or 2,4-D or left unsprayed.

Fungal filtrate was obtained by growing *Fusarium* on Dox' liquid at 25° C for 7 days; the resulting filtrate was distributed in test tubes, each received 15 cc of the fresh filtrate. Tubes containing similar amounts of either sterilized water or Dox' liquid served as controls. Dusting or spraying of cotton plants with either cotton dust or 2,4-D was achieved early in the morning. Some of the field-grown cotton plants, of both varieties, were dusted with cotton dust 24 hours before starting the experiment, using the usual way practiced for insect control. Some other plants were sprayed with a 20 p.p.m. solution of Fernoxone (commercial 2,4-D), this has been carried out 12 hours before starting the experiment. A third lot of field plants, far from the treated lots, was left untreated to serve as control. Both untreated and treated plants of more or less similar length were uprooted and brought as such to the laboratory. For every treatment and for each cotton variety, leaves of more or less similar age and area, were cut under water, dried and weighed. Their petioles were dipped in the test tubes containing either the filtrate, Dox' liquid or sterilized water. For each treatment four leaves were tested. After 24 hours dipping, leaves were removed, their petioles dried and reweighed. The percentage decrease was considered as a function of leaf wilting. The results could be summarized as follows (Table III):

TABLE III.

Percentage increase (+) or decrease (—) in the weight of cotton leaf of both varieties treated with either cotton dust or 2,4-D; leaf petioles are dipped either in Fusarium filtrate (F), Dox' liquid (D) or water (W).

Treatment of leaf	Criterion	"Giza 26" dipped in:			"Menoufi" dipped in:		
		(W)	(D)	(F)	(W)	(D)	(F)
Normal	Initial weight *)	1.95	2.37	2.23	2.30	2.15	1.99
	Final weight	1.99	2.39	1.25	2.32	2.21	0.98
	Percentage change	2 % (+)	1 % (+)	44 % (—)	2 % (+)	3 % (+)	51 % (—)
Cotton dust	Initial weight *)	2.30	2.31	2.30	2.09	2.29	2.10
	Final weight	2.30	2.38	1.22	2.05	2.27	1.16
	Percentage change	—	3 % (+)	47 % (—)	2 % (+)	1 % (—)	45 % (—)
2,4-D	Initial weight *)	2.09	2.28	2.09	2.22	2.00	2.04
	Final weight	0.82	0.89	0.67	0.80	0.70	0.78
	Percentage change	61 %	61 %	68 %	67 %	65 %	62 %

*) Average weight of 4 leaves.

a. Leaves previously treated with 2,4-D showed the maximal water loss. This applies to leaves of the two cotton varieties and to all dipping media. The wilt-inducing capacity of the filtrate was concealed by the more considerable wilt potency of 2,4-D.

b. The wilt response of either the susceptible "Giza 26" or the resistant "Menoufi" cotton variety towards *Fusarium* filtrate was more or less similar.

c. Previous dusting of leaves with cotton dust did not appreciably influence their wilting responses.

IV. DISCUSSION

Cotton dust has been extensively applied in Egypt for combating Cotton leaf worm. The significant accumulation of cotton dust constituents namely, DDT and BHC (SMITH, 1948 and FLEMING, 1950), with continual dusting in successive seasons, would influence the host-parasite relationship, a matter which would be taken into careful consideration. Although the annual application of cotton dust does not generally exceed 20 kilograms per acre i.e. 22 p.p.m. (assuming that the surface layer of an acre weighs 900.000 kilograms and provided that it is 20 cm in thickness and the weight of a cubic meter is 1070 kilograms) yet in the present investigation relatively much higher cotton dust concentrations are dealt with, in order to elucidate the following points: (1) The high local concentrations due to uneven dusting in field practice. (2) High concentrations as a result of the accumulative effect of the insecticide, due to the successive dusting in the following years. (3) The evaluation of cotton dust concentration which would, partially or completely, disturb the host-parasite relationship.

On the other hand very low concentration of 2,4-D was dealt with in the present investigation, as cotton-seed germination had been proved by the same authers (Unpublished work) to be highly sensitive to extremely low concentrations of 2,4-D and since actual fungus invasion takes place in the seedling stage, an experimental difficulty arose which necessitated the application of only low concentration of the herbicide, such low concentrations were found to have no appreciable effect on cotton-*Fusarium* relationship, probably it may cause a slight decrease to the normal susceptibility of "Giza 26" cotton variety.

With regard to *Fusarium* cotton-wilt, FIKRY (1932) isolated four species of *Fusarium* from diseased Egyptian cotton plants and identified by WOLLENWEBER as: (I) *Fusarium orthoceras* APP. et WR. (2) *F. vasinfectum* ATK. var. *indoratum* WR. (3) *F. angustum* SHERBAKOFF (4) *F. solani* (MART. pr. p.) APP. et WR. FIKRY stated that the first three *Fusaria* spp. were pathogenic while *Fusarium solani* was nonpathogenic. On the other hand, the present isolated *Fusarium solani* — identified by W. L. GORDON as *F. solani* sensu SNYDER & HANSEN — was proved to be pathogenic, its pathogenicity was, however, less than *Fusarium vasinfectum* which was used by the same authors in a previous investigation (MOSTAFA & GAYED, 1953).

Previous authors studying the effect of insecticides on host parasite relationship were only dealing with single insecticides namely, DDT (HEUBERGER & STREARNS, 1946, MACDONALD, 1947, JOHNSON, 1946, BOWSER, 1946 . . .) or BHC (YERSIN, 1947, BONDE SNYDER, 1946 . . .) or sulphur (FIKRY, 1936 & 1948, ZAUMEYER, 1946, BIOXO, 1947 . . .). The importance of the present investigation lies in the fact that it evaluates the combined effect of these three insecticides in the form of cotton dust. The latter proved to have no appreciable effect on cotton-*Fusarium* relationship.

SUMMARY

The pathogen *Fusarium solani* sensu SNYDER & HANSEN, as identified by W. L. GORDON was recorded in the present work as a new species causing cotton wilt in Egypt. Cotton dust in varying concentrations does not significally alter the normal infection of both "Menoufi" and "Giza 26" cotton varieties towards *Fusarium*. Similarly the potency of *Fusarium* filtrate to induce wilting did not appreciably change with previous treatment of cotton plants with cotton dust. On the other hand leaves treated with 2,4-D showed the maximal water loss. Percentage infected "Giza 26" cotton seedlings were found to be comparatively less in soil infected with 2,4-D treated *Fusarium* mycelium than in that infected with untreated mycelium.

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PRODUCTION OF CRYSTALS BY ROZITES GONGLYOPHORA. II - SOME PROPERTIES OF THE CRYSTALS. INFLUENCE OF THE TEMPERATURE AND OF THE CULTURE MEDIUM ON THE PRODUCTION OF CRYSTALS

by

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In a previous paper (Borzani, 1960) it was shown that *Rozites gonglyophora* cultivated in glucose agar, in banana solid medium, and in glucose broth with and without aeration, produces large quantities of crystals that remain between the hyphae: it was also shown that these crystals present some peculiar properties regarding solubility and high temperature resistance.

This paper has the purpose to present the results obtained in experiments carried out in order to study the crystals produced by *Rozites gonglyophora*, and the influence of the temperature and of the culture medium on the production of crystals.

Some properties of the crystals produced by Rozites gonglyophora.

It was already shown that temperature as high as 350° C only produces small crystal deformations (Borzani, 1960). The exposition of the crystals to the direct flame of a laboratory common burner during 12 minutes, also produces only deformations. These facts show that the crystals are probably of inorganic nature. The crystals are anisotropic when observed in polarized light. They are attacked by solutions of $AgNO_3$ and become black, probably by deposition of metallic silver. The crystals are also dissolved by solutions of $ZnSO_4$, $CuSO_4$ and $HgCl_2$, but are not attacked by solutions of KCl and NaCl.

Spectrographic analysis were made in materials obtained by three different methods: 1-mycelium carbonized by heating; 2-mycelium treated with HCl (approximately 20 %), centrifuged and the solution heated to dryness; 3-ashes of the mycelium (obtained by direct heating on Bunsen burner). Materials 1 and 2 presented aluminium in its composition.

The presence of aluminium was also verified by the following reactions:

1. **Quinalizarin reaction** (TREADWELL & HALL, 1937).-The mycelium of *R. gonglyophora* was treated with H_2SO_4 (4N) and the solution was submitted to the quinalizarin test with positive result.

2. **Aluminon reaction** (CHARLOT, 1949).-By means of microneedles and micropipettes, single crystals were isolated, treated with NaOH solutions and then with the aluminon reagent; in one case the reaction was positive. The difficulties of carrying out reactions in a microscopic scale can probably explain the failures observed with these tests.

We were not able, until now, to isolate the crystals from the mycelium in order to study its composition and properties.

INFLUENCE OF THE TEMPERATURE AND OF THE CULTURE MEDIUM ON THE PRODUCTION OF CRYSTALS

Cultures of *R. gonglyophora* maintained at temperatures of 15°C to 25° C approximately, produce large quantities of crystals. The fungus does not grow at 30—32° C, and is killed if kept at this temperature for 2—4 days. Temperatures as low as 4—5° C do not kill *R. gonglyophora* but do not permit its development.

From the original culture cited in our previous paper 21 sub-cultures on glucose agar (SALLE, 1954, p. 169) were obtained with approximately one month interval between two consecutive sub-cultures. In all these sub-cultures the production of crystals was observed.

Glucose agar mediums were inoculated with isolated hyphae of *R. gonglyophora*; after 15—20 days visible colonies appeared with production of large quantities of crystals.

These two last experimental observations, associated to the fact that the culture mediums do not present crystals, confirm that these crystals are produced by *R. gonglyophora*.

It was observed that, independently of the inoculum size, the colonies present a quite uniform development after one month incubation. This development can be evaluated in the following way: the medium of one or more tubes containing the fungus is melted, filtered in a gooch crucible, washed with hot distilled water, dried at 100—110° C for 3 hours and weighed; the result is calculated in mg per colony. The following results were obtained with one month old colonies grown on glucose agar: 20, 14, 22, 17, 17, 16, 15, 30, 17 and 17 mg per colony, with an average value of 19 mg per colony (per cent standard deviation of the average: 7.9 %); these measurements show that, in fact, the colonies after one month of incubation are practically uniform. This fact permits to establish a basis for the comparison of developments under different cultural conditions.

R. gonglyophora were inoculated on the following solid culture mediums, with production of crystals:

1. Agar (10, 15, 20, 25 and 30 g/liter).,
2. Gelatin (300, 350 and 400 g/liter).
3. Agar (25 to 30 g/liter) and one of the following materials: glucose, levulose, sucrose, xylose, arabinose, ribose, soluble starch and lactose (5, 10, 15 and 20 g/liter), meat extract (3 g/liter), peptone (5 g/liter), blackstrap molasse (sugar concentration calculated as glucose: 15 g per liter of medium).
4. Gelatin (400 g/liter) and one of the following materials: glucose, levulose, sucrose, xylose, arabinose, ribose, soluble starch and lactose (5, 10, 15 and 20 g/liter).
5. Agar (25 to 30 g/liter), glucose (15 g/liter) and one of the following materials: ammonium chloride, ammonium nitrate, ammonium acetate, peptone, glycine and alanine (2, 4, 6 and 8 g/liter), meat extract (3 g/liter).
6. Agar (25 g/liter), meat extract (3 g/liter) and peptone (5 g/liter).
7. Agar (20 g/liter), meat extract (3 g/liter), peptone (5 g/liter), glucose (10g/liter) and aluminium nitrate (0.5, 1.0 and 1.5 g/liter).
8. Hydrolised malt (hydrolysis at 45° C, followed by clarification and filtration) and agar (20 g/liter).

The fungus did not grow on media containing agar (30 g/liter), glucose (15 g/liter) and one of the following materials: sodium nitrite, sodium nitrate and urea (2, 4, 6 and 8 g/liter).

The media containing agar were sterilized by autoclaving (120° C; 15—20 min), while the gelatin media were sterilized by tyndallisation.

It was observed that the production of crystals is adversely affected by culture mediums that favour development. Thus, for instance, *R. gonglyophora* grows rapidly on malt-agar medium but produces only a few crystals.

R. gonglyophora inoculated on culture medium containing aluminium nitrate (concentration of 1.5 g/liter) produces very large crystals of about 60 μ of maximum length. The colonies cultured on other mediums never presented crystals greater than about 40 μ.

Cultivation on glucose broth (Salle, 1954, p. 170) by the method described by Santoro & Casida (1959) was also tried; the fungus grows rapidly but periodic agitation of the culture flasks do not permit the formation of large and well formed crystals. On stationary glucose broth, however, the fungus grows slowly and produces large and well formed crystals.

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CRYSTAL FORMATION IN CULTURES OF FUNGI:
Dermatophytes and other Hyphomycetes [1])

by

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(15.IV.1961)

(with 2 color-plates)

> "Crystals are the flowers of the mineral kingdom"
>
> ENGLISH and JENSEN

INTRODUCTION

Crystal formations in cultures of Hyphomycetes are with very few, most recent exceptions not recorded in the literature. Evidence for this statement is the fact that two well known texts, that of W. J. NICKERSON (1947) and of V. W. COCHRANE (1958) do not mention the existence of crystals in cultures of Hyphomycetes at all. Specifically medical mycological texts such as those of VANBREUSEGHEM (1952); of COUDERT (1955); of DA SILVA LACAZ (1956); of MOSS & MCQUOWN (1960); of CIFERRI (1960), etc., to mention only an important few, despite their most careful survey of the literature, have no cognizance of crystals, apparently for the simple reason because they could not find any data in the literature.

It is amazing that one had to go back almost eight decades into mycological literature to find anything about crystal formation in cultures of Hyphomycetes. ZOPF (1890) referred to the work of VAN TIEGHEM (1878) who found crystals in cultures of quite a number of Fungi Imperfecti. These structures were of two kinds: there were "crystalloids" and then, true crystals. In 1890 one defined "crystalloids" as crystals of protein nature as they occur within the cells of certain Phanerogams (e.g., in the protein particles of the cells of the Ricinus seed) and in cells of the red sea algae (Florideae). Crystalloids were considered as reserve foodstuff which eventually disappeared during the metabolic processes.

Occurrence of crystals was considered as a rarity in the world of fungi because their existence was, at that time (1890), known only in the Phycomycetes (Mucoraceae) studied first by KLEIN (1872) and later by VAN TIEGHEM (1878).

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[1]) Presented with demonstration at the mid-winter meeting of the Chicago Medical Mycological Society, February 15, 1961.

Van Tieghem clearly recognized that crystals in the Mucoraceae were true crystals, always extracellular, formed by the metabolites of these fungi.

There is a great jump from the early seventies of the nineteenth century to the mid-century of the twentieth to witness the appearance of conscious knowledge of crystal formations in fungus cultures. I could find but two publications of the recent past. One is Borzani's paper (1960) on production of crystals by *Rozites gonglyophora*. The other is the publication of Ahmadjian & Reynolds (1961). The latter found crystals in the culture of isolated lichenized fungi. In a culture of the mycobiont of *Cladonia cristatella*, in its late growth stage, abundant prismatic plate-like crystals were found in the regions of agar where the pigment had diffused and also on the hyphal cells. These crystals were insoluble in water and acetone and were found only on solid mediums.

SOME REASONS FOR THE LATE DISCOVERY OF CRYSTALS IN CULTURES OF HYPHOMYCETES.

Quite at the outset it seems to be useful to call attention to the fact that fungi do not "produce" crystals, such as a hen lays eggs. Fungi only produce certain metabolites on different culture mediums which, not being water soluble, crystalize. There are hyphomycetes as will be seen, whose metabolism leads to crystal formation only on solid, artificial medium such as Sabouraud glucose agar. Others may yield crystalizing metabolites on natural medium such as polished rice or in liquid medium such as glucose broth.

It is not out of place to reflect upon the circumstances which interferred with or prevented the discovery of crystals in fungus cultures. There is a number of factors.

1) As far as my experience extends at present, I have found that not all fungi produce crystalizing metabolites on any of the artificial, natural, solid or liquid mediums. Thus, they could not be found.

2) The culture may contain only a few crystals scattered throughout the culture which by an inexperienced eye and using ordinary light microscopy can be easily overlooked.

3) Certain clearing fluids may have a crucial inhibitory effect upon the visualization of crystals. Most unfortunately, the overwhelming majority of medical mycological texts still recommend as clearing fluid, potassium hydroxide (KOH) 10, 20, or even 40 per cent in strength. Even though most of the crystals are insoluble in alkali, however, when such a preparation is heated until the first bubbles appear, this high concentration of KOH suddenly crystalizes, covering up the true findings, the real fungus crystals. Crystalization may take place even without heating, in a few hours with destruction of the preparation.

In my writings I have always insisted that KOH should definitely be discarded and only chlorallactophenol should be used as a clearing or mounting fluid. Great institutions such as the Commonwealth Mycological Institute at Kew, Surrey, and many others, use this clearing fluid exclusively. It is a cold method (no heating!) and beautifully clears colored fungus particles, like mycelia, and permits the crystals in the mesh of mycelia to clearly stand out.

4) A vital handicap which causes the crystals to be overlooked is the use of ordinary light microscopy. True crystal "hunting" is possible only in polarized light. In polarized light no anisometric particle can be overlooked. Even crystal "dust" (crystals in the magnitude of pinpoint size under high, dry observation) will show up in brilliant colors at crossed nichols like stars in the Milky Way.

5) Those who never have had a course in crystallography in their college days will be most handicapped. Some may find difficulties in working with polarized light,¹) if not familiar with this technique.

However, all of these difficulties can be overcome. I whole-heartedly agree with ENGLISH and JENSEN that crystals belong to the most beautiful creation of nature. The beauty of their limitless, changing structures, their endless color combinations as they show up on dark background in polarized light, creates high, esthetic enjoyment. "Crystal hunting" may also lead to a peculiar "addiction" in that one regrets leaving the polarizing microscope. . .

THE PRESENT STUDY

(a) Materials and methods

After discovery of the presence of great number of crystals whose structures did not leave any doubt at first sight regarding their nature, in cultures of *Keratinomyces ajelloi* VANBREUSEGHEM on Sabouraud glucose agar (Difco Bacto Brand), - systematic examinations of cultures of Dermatophytes and other Hyphomycetes have been undertaken. Fungus growths have been examined for crystals growing on, 1) Sabouraud glucose agar (Difco Bacto Brand),2) polished rice and 3) Sabouraud glucose broth (Difco Bacto Brand). The technique is simple. A small particle of the fungus growth is mounted on a standard slide in chlorallactophepnol and observed, first in white light microscopy, then in polarized light,

Culture particles should be taken from different parts of the fungus growth because the crystals cannot be found in a single group. They are scattered throughout the culture. The age of the culture is not important. Crystals may be found as early as the seventh day. Yet, older cultures, of course, contain more crystals.

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¹) To work with polarized light, it is not necessary to have a special polarizing microscope. Any modern microscope for direct, white light can be transformed into a polarizing microscope with relatively modest expense. Most of the microscope manufacturers can provide special polarizing sets. Zeiss, for instance, has a very handy polarizing set, consisting of two nichols and a quartz wedge. The price is presently on the market at about $50.—.

There is a special reason for scanning the preparations both in direct, white light and in polarized light. Crystals occurring in fungus cultures may not always be anisometric (giving double refraction and the most varied, beautiful color combinations) but ofttimes isometric which are completely blacked out in polarized light. With their clear transparency these isometric crystals can only be visualized in direct, white light with, of course, narrowed diaphragm in the manner one observes unstained culture mounts.

Crystals in chlorallactophenol mounts, framed with clear, transparent nail polish can be preserved for a collection. Due, however, to the fact that chlorallactophenol may detract from the beauty and brilliance of the crystals in the long run, I suggest using simple distilled water as mounting fluid in preparations destined for collection.

Crystal formations can also be observed in hanging drop cultures. For this purpose I use either glucose agar or broth.

What one cannot observe is the "birth" of a crystal in a culture. In microchemical reactions I carried out, it could be observed that the transformation of an amorph material (e.g., calcium oxalicum) (Ca (C_2O_4). $3H_2O$) under the influence of sulfuric acid (H_2SO_4) into crystal forms takes place within a thousandth of a second or even less. In a culture, one is simply not "present" to witness this event.

LIST OF ILLUSTRATIONS[1]

A) DERMATOPHYTES[2]

1 2 3	*Keratinomyces ajelloi* VANBREUSEGHEM, strain No. 509
4 5 6	*Keratinomyces ajelloi* VANBREUSEGHEM, strain No. 208
7 8	*Keratinomyces ajelloi* VANBREUSEGHEM, strain No. 509
9	*Keratinomyces ajelloi* VANBREUSEGHEM, strain No. 210
10	*Keratinomyces ajelloi* VANBREUSEGHEM, strain No. 208
11 12 13	*Epidermophyton floccosum*
14	*Microsporon, n. sp.* (*Khartoum, Sudan*)
15	*Keratinomyces ajelloi* VANBREUSEGHEM, strain No. 208

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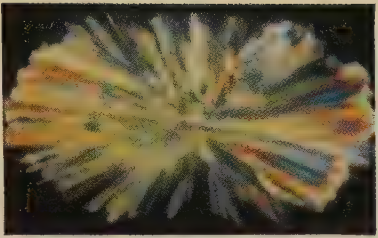
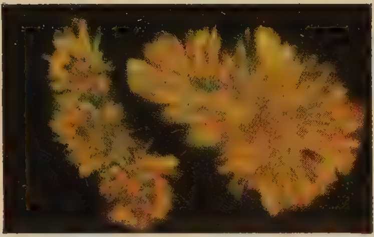
[1] All photomicrographs were taken by the author on Kodacolor film in polarized light. Photo data: Zeiss ocular 8x, objective 40x; afterwards linear magnification 2x. Light source: Zeiss microphoto lamp with 6 volt bulb.

[2] Where not otherwise indicated, all cultures were grown on Sabouraud glucose agar (Difco Bacto Brand). The age of the cultures varied from 7 days to several months.

1





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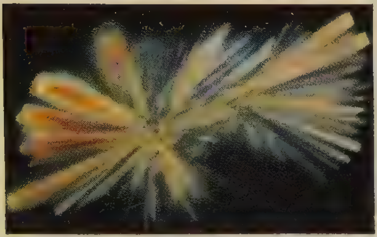

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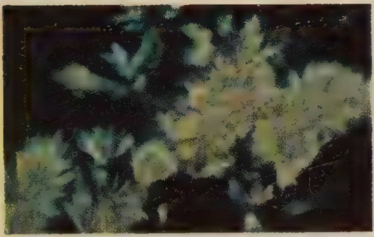

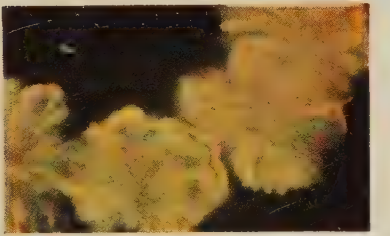

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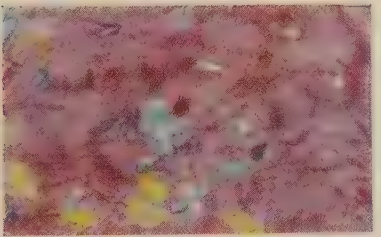

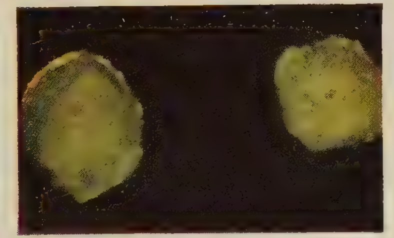

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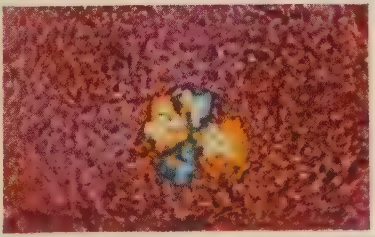

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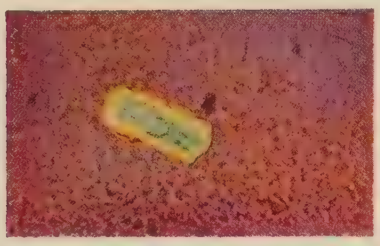

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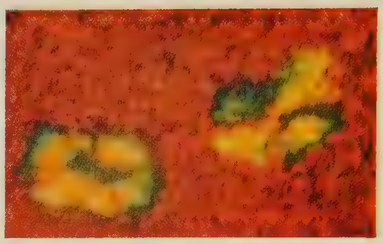

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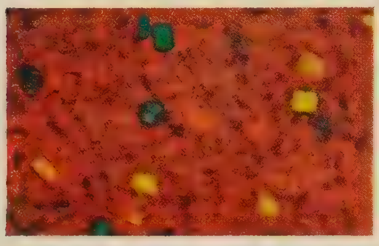

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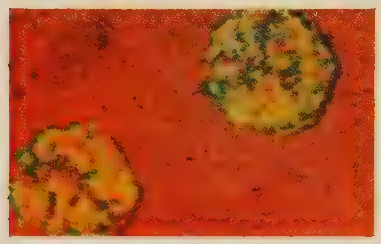

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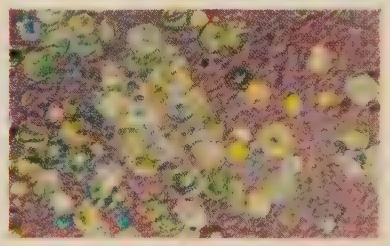

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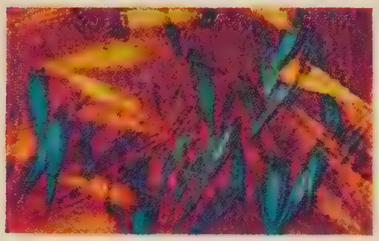

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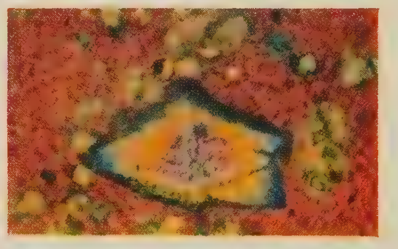

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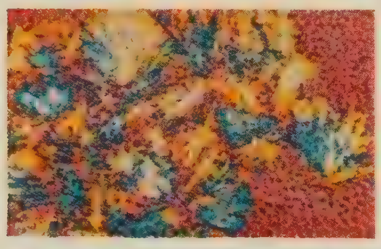

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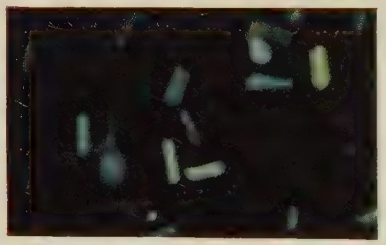

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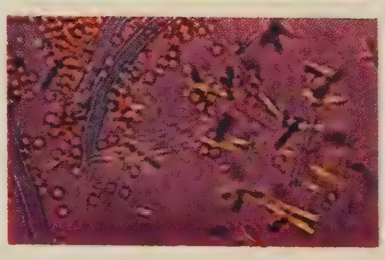

16 *Epidermophyton floccosum*
17 *Keratinomyces ajelloi* VANBREUSEGHEM, strain No. 508
18 *Keratinomyces ajelloi* VANBREUSEGHEM, strain No. 208 (from broth)
19 *Microsporon audouinii*, strain No. 211
20 *Keratinomyces ajelloi* VANBREUSEGHEM, strain No. 508

B) OTHER HYPHOMYCETES [2])

21 *Mixotrichum conjugatum* (on rice)
22 *Rozites gonglyophora* [3])
23 *Aspergillus fumigatus*
24 *Aspergillus flavus*

(b) The crystal formations

The basic aim of the present study is to show the great variety of crystal forms as they occur in fungus cultures. The different strains of the same species of fungi may produce the most different crystallographic forms. (Cf., crystal formations in cultures of *K. ajelloi*). On the other hand, the same crystallographic forms may occur in cultures of different, unrelated species of fungi. (Cf., Figs. 12 and 14, *Ep. floccosum* and *Microsporon* n. sp. (Khartoum, Sudan), respectively.) Despite the identical chemical composition of the crystals, their crystallographic forms may show the greatest variety. One substance, the calcite ($CaCO_3$), for instance, at the identical chemical composition, may have 300 different crystallographic forms. The crystal forms may be entirely different in the same species, as to whether they grew on solid or liquid mediums. (Cf., Figs. 15 and 18 ,*K. ajelloi* on solid and liquid mediums, respectively.)

In the language of crystallographers, the crystals may be acicular (very slender forms) (Fig.9); capillary (Fig. 11); radiated-stellate (Fig. 10); columnar (Figs. 13 and 22); rosette (Figs. 1,2,3) and, many other forms.

In the present study crystal formations are offered in the following fungus species and/or strains:

Keratinomyces ajelloi,	4 strains
M. audouinii,	1 strain
Ep. floccosum,	1 strain
Microsporon n. sp. (Khartoum, Sudan),	1 strain
Mixotrichum conjugatum,	1 strain
Rozites gonglyophora,	1 strain
Asp. fumigatus,	several strains
Asp. flavus,	several strains

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³) I am indebted to Prof. W. BORZANI, São Paulo, Brazil, who kindly placed at my disposal a culture of the fungus, *Rozites gonglyophora*. The metabolites of this fungus yielded a huge number of beautiful crystals. I can confirm the findings of Prof. BORZANI.

SUMMARY

The present study calls attention to crystal formations in cultures of Dermatophytes and other Hyphomycetes.

The reasons for the late discovery of crystal formations in fungus cultures are discussed.

The diverse crystallographic forms found up to date in cultures of fungi enumerated are described and illustrated.

ZUSAMMENFASSUNG

Die gegenwärtige Untersuchung lenkt die Aufmerksamkeit auf die Krystallbildung in Kulturen von Dermatophyten und anderen Hyphomyceten.

Die Gründe für die späte Entdeckung von Krystallbildungen in Pilzkulturen werden erörtert.

Die verschiedenen krystallographischen Formen, die bisher in Pilzkulturen gefunden worden sind, werden beschrieben und illustriert.

RÉSUMÉ

La présente étude informe sur la formation des crystaux dans les cultures des dermatophytes et d'autres hyphomycètes.

Les raisons pour la découverte tardive des formations des crystaux dans les cultures des cryptogames sont discutées.

Les diverses formes crystallographiques trouvées dans les cultures cryptogamiques ont été décrites et illustrées.

ACKNOWLEDGEMENT

It gives me great pleasure to acknowledge the receipt of a grant from Dome Chemicals, Inc., New York City, and to express my gratitude to its president, Mr. IRVING B. WERSHAW who alone made it possible to publish this crystal study in color.

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A HISTOPATHOLOGICAL STUDY OF EXPERIMENTAL MURINE SPOROTRICHOSIS

by

MASAHIKO OKUDAIRA, EIRO TSUBURA & JAN SCHWARZ[1])

(with 10 figs.).

(23.XII.1960)

In a previous report (1) the therapeutic value of griseofulvin and amphotericin B in experimental systemic murine sporotrichosis was evaluated. The present study deals with the microscopic features of the lesions in mice with experimental sporotrichosis and attempts to answer the following questions:

1) What is the natural course of experimental systemic sporotrichosis in mice? Which organ shows the most extensive alterations? Is there any difference in the tissue reaction in the various organs? Is there any relationship between the tissue reaction or experimental course and the structure of the micro-organisms in the tissue phase?

2) What are the microscopic alterations induced by treatment with amphotericin B and griseofulvin.

MATERIAL AND METHODS

Female mice of approximately 20 g weight were injected with a million cells of the yeast phase of *Sporotrichum schenckii* as described previously (1). Tissue preserved at necropsy was fixed in 10 % formalin. Paraffin sections were prepared and stained with hematoxylin and eosin, and by the Gridley and Grocott procedures.

Histologic lesions in the liver, heart, kidney and the spleen were compared. The contour of *Sporotrichum schenckii* in its tissue phase was drawn with the Abbe camera lucida.

RESULTS

Histologic features in different Organs.

The intensity of the lesions varied from mouse to mouse, even those which died on the same day. In each experimental group, however, the histologic features had a recognizable tendency: i.e. a sequence of pathologic changes paralleling the number of days after inoculation. (Table I).

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[1]) From the Clinical Laboratories, Jewish Hospital, Cincinnati and the Laboratory of Mycology (Department of Dermatology and Pathology) College of Medicine, University of Cincinnati. The project was in part supported by Grant E-576 from the National Institutes of Health and the Brown-Hazen Fund.

A. *Control group* (*No treatment*)

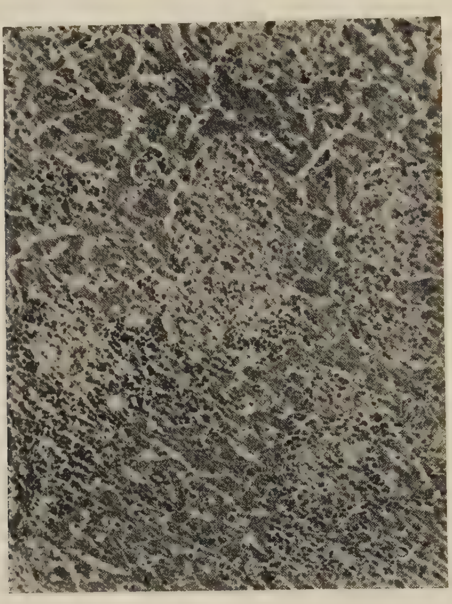
a. Liver: The mice that died at or about 2 weeks after inoculation showed many small or medium sized lesions scattered throughout the liver. The lesions were distributed in the lobules and in and around the portal areas. They consisted chiefly of single phagocytes containing a number of organisms. With hematoxylin and eosin stains the organisms were faintly basophilic, rodlike or dotlike, measuring 3 to 4 μ by 1 to 2 μ. Each was surrounded by a remarkably clear halo which averaged 1 to 2 μ in width. In sections stained by the Gridley or Grocott method most of the organisms were spindle or cigar-shaped, measuring 5 to 8 μ by 2 to 4 μ and were distributed densely in the lesions. Ovoid or round forms were scarce at this stage. (Photos 1 and 5).

Some of the lesions showed central necrosis in the sporotrichotic lesions. There was no inflammatory cell infiltration in and around the intralobular lesions. Those in the portal areas were essentially similar except that they were often accompanied by histiocytic infiltration. Portal and hepatic vessels exhibited swollen endothelial cells; their muscle fibers were thickened and separated by histiocytic and neutrophil infiltration. Hepatic cells situated about the lesions showed atrophy and degeneration due to compression. Some parenchymal cells also contained rodlike organisms. The boundary between the lesions and hepatic cords was usually well defined. Kupffer cells appeared to have proliferated. Some were remarkably swollen and contained numerous organisms.

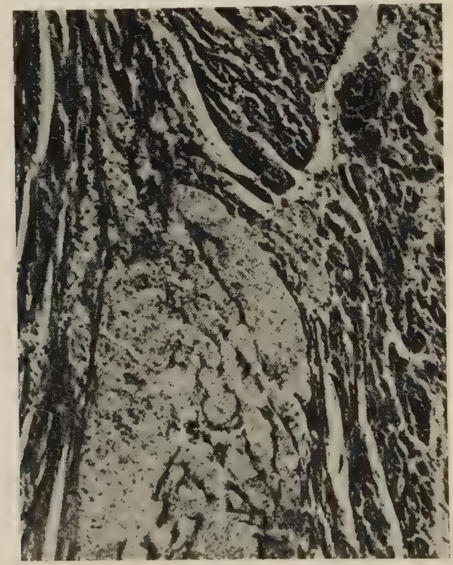
At 3 weeks the lesions had a tendency to enlarge and become confluent. There were increased numbers of clustered Kupffer cells containing organisms. Periportal histiocytic infiltration was more prominent. Some of the lesions situated beneath the hepatic capsule had spread to produce a focal perihepatitis. The peritoneal epithelium had proliferated and contained numerous organisms. The actual number of organisms was almost the same as in the "two week group" and pleomorphism was not an outstanding feature at this stage.

At 4 to 5 weeks the lesions became smaller. The balloon-like appearance of the phagocytes was less prominent. There was a striking decrease in the number of recognizable organisms. Central degeneration and necrosis of the lesions had subsided at this stage. Histiocyte, plasma cell and lymphocyte infiltration in and around the lesion was more conspicuous than in the early stages. Giant cells were observed in two cases. Although the total number of organisms had decreased, ovoid and round forms of the organisms were more frequent and pleomorphism was more conspicuous. The "cigar bodies" were reduced in size measuring 5 to 6 μ by 1.5 to 2 μ and ,,ring forms" from 1.5 × 1,5μ to 8 × 10 μ.

Plate I.



1. Control (17 days). Many medium sized sporotrichotic lesions are scattered throughout the liver. Hematoxylin and eosin stain, × 60.



2. Control (18 days). Aggregation of phagocytes containing numerous organisms, are seen in the interstitium of the myocardium. Hematoxylin and eosin stain, × 60.

b. Heart: At 2 weeks, there was an increased number of perivascular histiocytes but no other noticeable alterations. A few organisms, almost all of which appeared as "cigar bodies" were found in the perivascular spaces.

At 3 weeks, perivascular accumulations of phagocytes, containing numerous organisms were seen. The nuclei were elliptical to irregular in shape and rich in chromatin content. Arterioles exhibited slight endothelial swelling and marked leuco-erythrostasis. There were conspicuous myocardial changes in the form of cloudy degeneration of fibers and organisms were very numerous and partially found in myocardial fibers. (Photo 2).

At 4 to 5 weeks, lesions were increased in both number and size. Pericarditis and endocarditis were often manifest due to extension from the myocardial lesions. The organisms in the histiocytes were less numerous, and more ring forms were noted. Scanty leucocytic and lymphocytic infiltrations were evident in and around the lesions. In these regions, myocardial fibers had undergone degeneration. Lesions were more frequently observed in the left ventricular wall and septum.

The pleomorphism of the organisms was, on a whole, less conspicuous in the heart than in the liver.

c. Kidney: Histologic changes in the kidney appeared in 2 forms. 1) Periarterial mononuclear cell infiltration in relation to arcuate and interlobular arteries. There were also perivenous lesions accompanied by thrombophlebitis. 2) Cortical lesions about the glomeruli. No medullary alterations were observed.

At 2 weeks, periarterial infiltration consisted chiefly of histiocytes and a few plasma cells, leucocytes and lymphocytes. Microorganisms were hardly detectable. In contrast, the perivenous phagocytes collected about veins, contained large numbers of organisms. Both periarterial and perivenous lesions usually accompanied each other. Glomerular lesions were not remarkable at this stage. However, a few collection of cells, chiefly phagocytes containing numerous organisms, appeared in the peritubular stroma and caused compression and atrophy of the tubules. The number of organisms in the kidney was small at this stage. They consisted chiefly of "cigar bodies" which were somewhat smaller than in the liver and heart. No casts were seen in the tubules.

At 3 weeks, perivenous lesions became more conspicuous and verrucous sporotrichotic lesions projected into the venous lumen in some areas. Periarterial infiltrations were less noticeable than a week earlier. There were scattered lesions throughout the cortex and various degrees of glomerular alterations were noted. Some glomeruli contained a few organisms, others contained many. In the latter instance there was distortion of the glomerular architecture with enlargement to twice or 3 times normal size. Stromal changes were more prominent and fungal casts were found occasionally. (Photo 3).

At 4 to 5 weeks periarterial infiltrations were considerably reduced and perivenous lesions were less common than in the early stages. Thrombi consisting chiefly of neutrophils were found in the venous lumens. The cytoplasm of the neutrophils was swollen with organisms. Ring forms were much more abundant than the "cigar bodies" and the organisms were markedly pleomorphic.

d. Spleen: 2 weeks after inoculation the spleen was moderately congested and there was hyperplasia of reticulum cells in splenic follicles. The reticulum cells contained a moderate number of organisms; some of their nuclei were pyknotic and nuclear debris was found in the center of the lesions. Lymphocytes were increased in number in the pulp cords. Although sinusoidal lining cells were swollen, they contained no organisms. The organisms in reticulum cells were mostly "cigar bodies" and appeared smaller than those in the kidney. Anemic infarctions were noted in a few instances.

At 3 weeks hyperplasia of the reticulum cells was more marked. Small amounts of nuclear debris were still present in some lesions. Viable reticulum cells were swollen and contained large numbers of organisms. Their nuclei were deformed and pyknotic. (Photo 4).

At 4 to 5 weeks reticulum cell hyperplasia was very marked; almost the entire splenic substance was replaced by these cells. Fewer organisms were demonstrated and ring forms were more abundant than "cigar bodies". Two types of ring shaped organisms were observed. One was a round body with a strong reaction to the periodic acid Schiff (PAS) stain throughout the fungus cell; the other was characterized by a thin wall.

B. Tissues from animals treated with griseofulvin or amphotericin B.

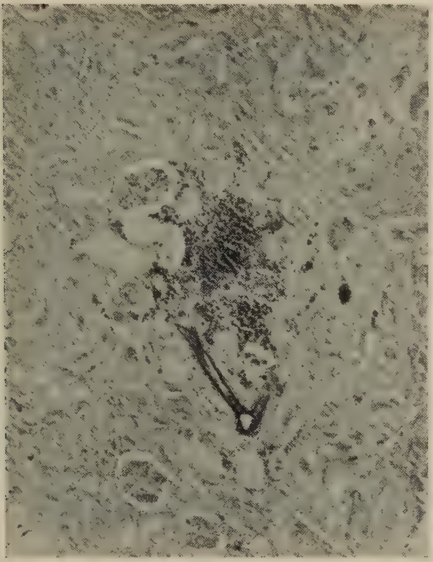
Organic lesions after the treatment with griseofulvin were identical with those described in the control groups. This was noteworthy because 5 different dosage levels were given. None of them influenced the pathologic alterations in any way.

The 2 groups treated with amphotericin B differed very strongly. The lesions in the mice, which received only one milligram per kilogram per day, were markedly different from the controls. Their features may be summarized as follows:

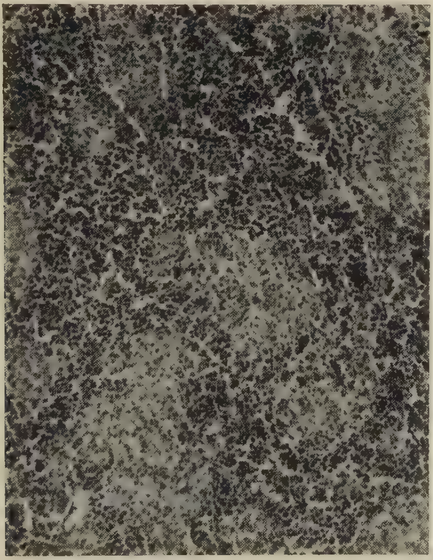
The liver contained much less inflammatory exudate and both periportal infiltration and phlebitis were of less intensity. On the other hand, the number of organisms observed was almost the same as in the control animals. However, there were more of the ring shaped organisms in the later stages; some of these measured as much as 10 μ.

In the heart there was little abnormality for approximately 2 weeks, but small lesions appeared at about 3 weeks. These were composed in the main, of histiocytes, which had ovoid organisms. In 4 to 5 weeks there was unmistakable involution and fibrosis apparent. The number of organisms also decreased sharply. Ring forms were more apparent than cigar bodies and in some cases myce-

Plate II.



3. Control (20 days). Small numbers of organisms in the periglomerular cortical stroma of the kidney. Gridley stain, × 60.



4. Control (20 days). Nodular sporotrichotic lesions in splenic follicles. Hematoxylin and eosin stain, × 60.

lial forms were observed. Hyphae found in the left ventricle consisted of septate elements measuring 2 to 3 μ in width, and some fragments measured as much as 50 μ in length. Positive cultures for *Sporotrichum schenckii* made it certain that the hyphae really represented the fungus under discussion.

Spleen and kidney showed only minor differences from the control animals.

In animals treated with 10 milligrams per kilogram per day of amphotericin B the alterations were completely different. In the organs examined, there were very few or no organisms. Special stains were necessary to demonstrate the few fungi present, and in many cases even prolonged search proved to be fruitless.

In the liver, granulomatous foci were moderately numerous in the first few weeks. After 4 weeks, there was a tendency toward involution. Kupffer cells still appeared disturbed, but no organisms were visible in hematoxylin and eosin stain preparations. With special stains, organisms were detected in only 6 of 20 cases. The organisms were scarce and most of them were ring forms. Their size varied from 1 to 8 μ; their staining features with Gridley and Grocott stains were much less intense than in the control cases.

The most conspicuous difference was found in the myocardium. The marked interstitial infiltration observed in the controls was absent and of 25 mice examined, 24 were free of organisms, even with special stains. The one heart which showed a few organisms was in a mouse which died thirteen days after inoculation.

In only one case were large organisms detected in the kidney; this too was in marked contrast to the control animals. In two cases occasional ring form organisms were seen in the spleen.

The morphologic features observed may be summarized as follows:

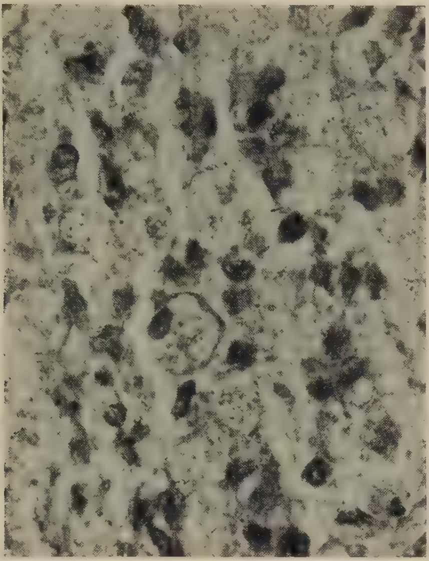
"Cigar bodies" predominated in the first 2 to 3 weeks after inoculation; ring forms appeared after 4 to 5 weeks. "Cigar bodies" were especially abundant when many organisms were found. On the other hand, a paucity of organisms was associated with ring forms.

Ring forms were of 3 different morphologic types. One was a round body which stained intensely and homogeneously with the PAS stain; they appeared during the early and late stages of the experiment. The second form, with a round body and a prominent central mass which stained darkly, was seen especially in the last stages of the cycle. The third type was a round body with a thin wall, and it also appeared mostly in the late stages. (Photos 6 and 9).

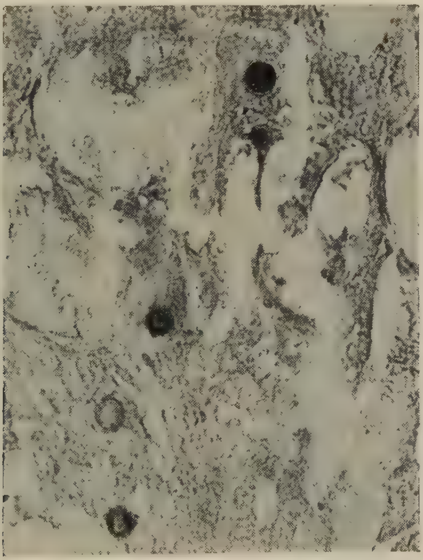
"Cigar bodies" stained well with hematoxylin and eosin. In 2 or 3 weeks the rod-like bodies became faintly basophilic but became slightly acidophilic after 4 to 5 weeks. In contrast, the ring forms hardly stained at all with hematoxylin and eosin.

The variation in size and shape of the organisms increased with

Plate III.



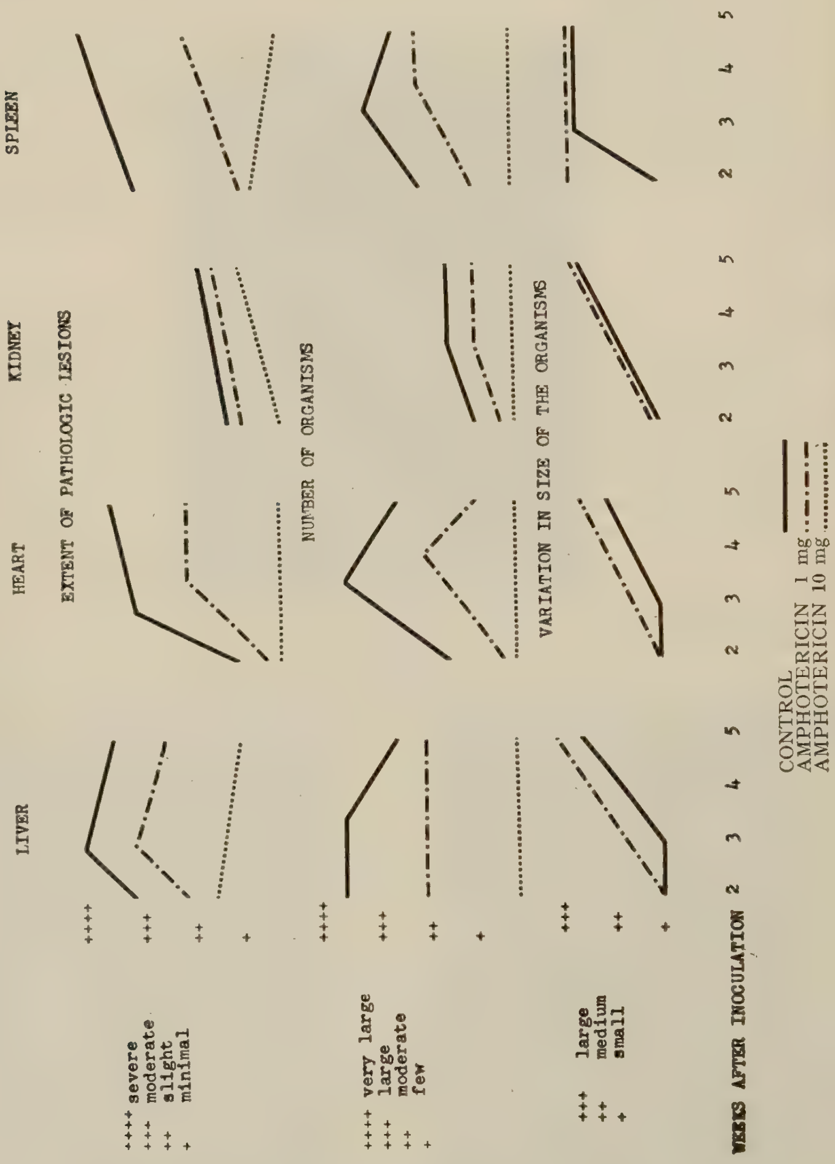
5. Control (17 days). Sporotrichosis, liver. The organisms are rod-like in shape and exhibit a clear halo. Hematoxylin and eosin stain, × 300.



6. Griseofulvin 75 mg (18 days). Kidney. Thick walled ring forms contain a central dark stained mass and are thin-walled. Gridley stain, × 300.

TABLE I.

Effect of Amphotericin B in Experimental Sporotrichosis



Excellent and detailed reports have been published concerning the tissue phase of *Sporotrichum schenckii*. SIMONS et al (6) described the life history of the organisms in rat tissue in the following manner; a) cigar body, b) early cryptococcal body, c) cryptococcal body, d) early asteroid body and e) asteroid body. MARIAT et al (8) identified the tissue form of the organism in an almost identical manner.

We ourselves, have observed great variation in the size and shape of the organisms in tissue sections in relation to the organ involved and the experimental conditions effected. In our studies, early asteroid bodies were not observed, probably because of the short experimental period. The "cigar bodies" or spindle shaped organisms were found in the early stage particularly (about 2 weeks); ovoid organisms with budding and early cryptococcal or round forms with thin walls were encountered in the later stage (4 to 5 weeks). An additional feature of significance was the appearance of hyphal elements.

It has been stated that the mycelial form of *Sporotrichum schenckii* has not been encountered in tissue (9); this is not entirely the case as the hyphal form has been described in one human case(10). No example of hyphal pattern has been described previously in the experimental disease, however. The *Sporotrichum* exhibits mycelial growth when cultured at room temperature. The transformation from ring forms to hyphal elements was apparent in our experimental material. The possibility of extraneous debris and other mycotic infections was excluded by morphologic and cultural investigation.

The mechanism of the hyphal form cannot be fully explained, but seems to be based on the influence of the antifungal agent used.

The therapeutic effectiveness of amphotericin B in experimental sporotrichosis was manifested by a paucity or complete absence of organisms in the mice treated with amphotericin B 10 mg/kg/day. Amphotericin B 1 mg/kg/day proved to be an insufficient dose since persisting lesions were found in most organs. The therapeutic action of amphotericin B in experimental sporotrichosis is in contrast to that of potassium iodide. The latter stimulates the healing process without inhibiting the development of *Sporotrichum* (2). Contrary to the results reported by GONZALEZ-OCHOA (11), griseofulvin treated mice showed no morphologic improvement in our experiment.

No unusual tissue reaction was observed in the treated groups although pleomorphism of the organisms was a more conspicuous feature.

SUMMARY

1. A histologic study of experimental sporotrichosis in mice was carried out. The reaction of tissue and the structure of the tissue phase of the organism in untreated controls and in mice treated with amphotericin B and griseofulvin were compared.

2. Considerable differences existed in the lesions and the number of organisms in the different organs; there was also variation in relation to the duration of the experiment.

3. Hyphal elements in the tissue in experimental sporotrichosis were observed for the first time.

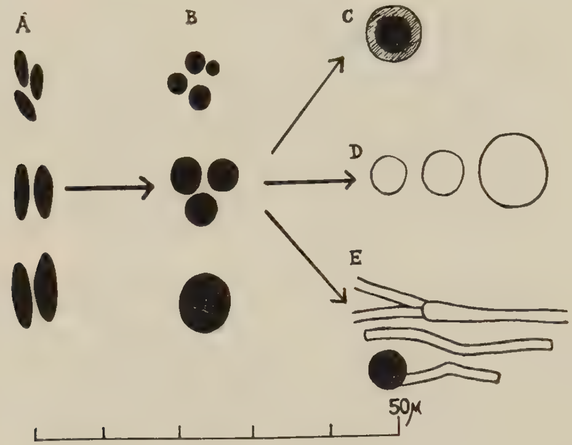


Fig. 1.
Sporotrichum schenckii in murine tissues.
Sketch indicates variations observed following intraperitoneal inoculation of the yeast phase in mice.

A: Cigar bodies
B: Ring forms
C: Ring forms with central mass
D: Ring forms with thin wall
E: Hyphal pattern

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SULLA PATOLOGIA SPERIMENTALE DA *ACTINOPLANES PHILIPPINENSIS* COUCH

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L'A. philippinensis Couch è un peculiare Actinomicete appartenente al genere *Actinoplanes*, alla famiglia Actinoplanaceae del l'ordine delle Actinomycetales. L'isolamento di questi actinomiceti, aventi alcune caratteristiche degli *Streptomyces*, ma riproducentisi per spore mobili (in quanto dotate di flagelli) formantesi entro sporangi, venne fatto quasi accidentalmente dal Couch (1949) su culture di foglie sterili quali egli usava per l'isolamento di muffe acquatiche e di Chytridiales. Il genere *Actinoplanes* proposto da Couch nel 1949, fu da lui descritto nel 1950, e quindi studiato con maggiore dettaglio dallo stesso micologo nel 1954. Nel 1955 Couch propose la famiglia delle Actinosporangiaceae, il cui nome fu quindi modificato in Actinoplanaceae (1955).

Alla famiglia Actinoplanaceae, che venne inserita nel Manuale del Bergey (1957) al quarto posto nell'ordine delle Actinomycetales, dopo le Mycobacteriaceae (I) le Actinomycetaceae (II), le Streptomycetaceae (III), appartengono due generi: I) il genere *Actinoplanes*; II) il genere *Streptosporangium*, dei due generi l'*Actinoplanes*, essendo il più comune ed interessante, è stato scelto come tipo della famiglia. Le caratteristiche fondamentali del genere *Actinoplanes* sono: micelio vegetativo, solitamente poco abbondante, si forma in acqua su particolari varietà di piante, anche però su terreni nutritivi ed allora colorato brillantemente dal rosa arancio fino al bruno scuro. Micelio aereo, di norma assente, se presente molto simile a quello degli *Streptomyces*. La riproduzione avviene per spore (zoospore) formantisi entro sporangi. Le spore sono munite di più flagelli e mobili.

Tipo e sola specie del genere *Actinoplanes* è l'*A. philippinensis*, isolato dal suolo nelle Isole Filippine, poi pure dal suolo e dall'acqua corrente vennero isolati successivamente altri 120 ceppi (1955) raggruppabili in più specie.

Le caratteristiche dell'*A. philippinensis* sono: colonie dure, date da ife densamente intrecciate, da convolute ad inoltrantisi nell'agar, in dense serie parallele a palizzata, di colore brillante, variabile da arancione a marrone secondo il substrato di cultura e l'età, e da lisce a cerebroidi. Micelio aereo assente o scarso, e comunque breve ed irregolare, con ife ramosette scarsamente ed irregolarmente septate, erette o sub-erette, anche aggregate ma lassamente, intricate o no. Gli sporangi, sferici o sferiodali, a parete sottile, granulosi prima della maturità, si formano nella porzione aerea delle ife, e, di solito, sono addensati alla superficie del plectenchima a palizzata. In una goccia d'acqua divengono meno rifrangenti e si forma un poro attraverso il quale sono eiettate le zoospore. Tali zoospore sono piccole, da sferiche ad elittiche, con un globulo rinfrangente centrale, in parte non mobili, in parte mobili subito, o dopo qualche tempo. I flagelli sono difficili da evidenziare anche con colorazioni elettive. In media sembrano due, di uguale lunghezza od uno più corto ed uno più lungo, ma vi sono zoospore anche con uno o tre flagelli. Dopo qualche ora di natazione, le zoospore si arrestano, i flagelli scompaiono e non si ha ulteriore evoluzione, oppure si forma un'ifa germinativa precocemente ramosa, ma a debole sviluppo. Nelle culture i conidi aerei sono pochi e irregolarmente prodotti, generalmente senza spirali o appena con accenni di ife vagamente spiralate.

La morfologia generale è quella di un Attinomicete aerobio, con micelio variabile, denso nella profondità dell'agar e scarso come micelio aereo, con poche spore aeree o senza, del tipo Streptomycetaceae. Più particolarmente la morfologia è quella del III gruppo delle *Nocardiae* con poco micelio aereo, crostosità delle colonie, etc. del tipo *N. asteroides* (McLung, 1949 et passim) con termini di passaggio al gruppo II che sembra più vicino a *Streptomyces*.

Seguendo alcuni punti di vista di R. Ciferri circa le affinità di questi Actinomiceti con alcuni gruppi di Cytridiales, abbiamo voluto indagare la possibile patogenicità sperimentale dell'*A. philippinensis*, scelto quale tipo del genere *Actinoplanes*, in animali di laboratorio.

Tale indagine ci è sembrata dovesse rivestire un certo interesse sia perchè non risulta sia stata finora espletata, sia perchè *l'Actinoplanes* si collega, sia pure alla lontana, con altre famiglie dell'ordine Actinomycetales comprendenti alcune specie sicuramente patogene per l'uomo e per gli animali (quali *A. istraeli*, *A. bovis*, *Nocardia asteroides* etc.) sia infine perchè il genere *Actinoplanes* è ubiquitario nel suolo e nell'acqua corrente. Contro ogni aspettativa, data la sua vita saprofitica in natura, l' *A. philippinensis* si è rivelato nettamente patogeno per il ratto albino (ciò che ci indurrà a continuare le ricerche anche per il genere *Streptosporangium*).

In questo primo contributo riferiamo quanto concerne l'attività patogena dell'*A. philippinensis* in relazione ai reperti istopatologici.

MATERIALE DI STUDIO E METODI

Il ceppo di *A. philippinensis* da noi usato per la sperimentazione è un trapianto dell'originale di COUCH gentilmente inviatoci da questo studioso fin dal 1957. Poichè a distanza di tempo, ci era sorto il dubbio che in cultura si potesse essere modificato, lo stesso COUCH ha acconsentito ad inviarci altre culture di comparazione che non hanno differito da quelle da noi conservate. Infatti la morfologia delle colonie non è variata e ne precisiamo la forma ed il colore sui vari substrati quale da noi osservati durante il periodo sperimentale.

Su agar patata glucosata: dopo $1\frac{1}{2}$-2 mesi a T. A. ed a 30° C ed a pH=7 colonia cerebroide plicata, sopraelevata al centro, di 18-20 mm di diametro, con solchi degradanti verso i margini lisci aderenti all'agar. La superficie della colonia appariva dapprima pastosa poi lucente di un colore variante dall'arancio brillante (¹) (tangerine orange) al vermiglio (vermillon) che poi, mano a mano, assumeva tutte le gradazioni dal color mogano (mahogany) fino al rosso bruno del Malaga (malaga) ed al baio (bay). La colonia produceva un pigmento diffusibile nell'agar.

Su terreno di Czapek, per lo stesso periodo di osservazione ed alle stesse condizioni di temperatura, si ebbe crescita meno rigogliosa: la colonia cerebroide, sopraelevata al centro, traslucida ad ampi margini plicati e sinuosi, fortemente aderenti al terreno, dava soprattutto una crescita in profondità. Il colore variava dal color cuoio chiaro al bruno fulvo (tawny) fino al castagno (tan) ed al ferrugginoso (russet brown) nelle colture più vecchie, con talvolta margini più chiari. Su terreno di Krainsky (dextrose asparagine agar) l'aspetto morfologico della colonia non differiva molto dalle precedenti, salvo che per le plicature della zona centrale più delicate, degradanti verso i margini. La superficie appariva lucente, traslucida di un colore variante dall'ocraceo (ochre yellow) al salmone (salmon), al camoscio (chamois) con margini più chiari.

Le culture su foglie di erba sterilizzata diedero risultati del tutto sovrapponibili a quelli descritti da COUCH.

Per la nostra sperimentazione ci siamo sempre serviti di culture lasciate crescere per due mesi su agar patata glucosata a pH=7,2 a 30° C.

Poichè alcune prove orientative preliminari da noi compiue avevano messo in evidenza la patogenicità dei ceppi di *A. philippinensis*, si è passati ad una sperimentazione su più vasta scala, servendoci di 23 ratti albini maschi del peso oscillante fra i 120-180 g. suddivisi in quattro gruppi a seconda della via di inoculazione.

I) gruppo di n. 12 animali inoculati per via intraperitoneale; II) gruppo di n. 5 animali inoculati per via endovenosa (vena dorsale del pene) III) gruppo di n. 2 animali inoculati per via intrates-

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¹) Colori secondo la Standard Chromotaxia di LOCQUIN (1957).

ticolare monolateralmente; IV gruppo di n. 4 animali inoculati per via intracranica (infissione profonda di ago da siringa attraverso la teca della volta). I dati relativi sono riportati nella Tabella.

Ogni gruppo di animali venne inoculato con sospensioni della stessa cultura. La cultura veniva ripresa in pochi ml. di soluzione fisiologica sterile e frantumata in mortaio sterile onde ottenere una sospensione omogenea. La densità della sospensione è stata mantenuta costante per tutta la durata della sperimentazione.

Ogni animale venne pesato all'inoculazione e prima del sacrificio; pei ratti morti spontaneamente il peso finale venne controllato prima della zoopsia. Per gli animali del gruppo II, inoculati per via endovenosa, venne eseguito anche un controllo del peso a quattro giorni dall'inoculazione. Per ogni lotto di ratti si procedette a sacrifici scaglionati nel tempo, per poter meglio seguire l'evoluzione dei processi patologici. Per tutti gli animali sacrificati si procedette alla zoopsia entro mezz'ora dall'uccisione. Per quelli morti spontaneamente entro il più breve tempo possibile. Durante la zoopsia da tutti gli organi presentanti alterazioni macroscopicamente rilevabili furono eseguite retroculture su agar patata a pH=7 e tenute poi a 30° C. Per l'indagine istologica vennero sistematicamente compiuti prelievi dai vari organi. I pezzi, prelevati in doppio vennero in parte fissati in formalina, in parte in Bouin alcoolico ed inclusi in paraffina. I metodi di colorazione usati furono: l'ematossilina-eosina, il metodo di Gram, il metodo di Hotchkiss (PAS). Non si ricorse al metodo di Ziehl, non essendo i filamenti dell'actinomicete allo studio acido-resistenti.

Reperti

Reperti macroscopici

A seconda della via d'inoculazione si sono potuti rilevare reperti patologici a carico di determinati organi nella totalità degli animali, mentre altri quadri sono strettamente legati alle peculiari modalità d'infezione.

Pressochè costantemente interessati da lesioni aspecifiche sono apparsi i grandi organi parenchimatosi, fra i quali maggiormente colpiti fegato, milza e reni, indifferentemente nei diversi gruppi di animali.

In rapporto alle varie vie d'inoculazione si è invece potuto apprezzare un elettivo interessamento di determinati organi, secondo le caratteristiche seguenti.

In molti animali inoculati per via endoperitoneale la sierosa del peritoneo si presenta diffusamente iperemica con scarse pseudomembrane; in corrispondenza del pancreas e dei legamenti pancreatico-lienale, gastro-epatico, epato-duodenale, gastrocolico e duodenocolico vi è una disseminazione di noduli di grandezza variabile da un grano di miglio ad un pisello, di colorito giallastro o bianco giallastro, spesso determinanti retrazioni ed addensamenti epiploici. In un caso, in uno dei due animali sacrificati in quattordicesima giornata (R/10), fu notata la presenza di un nodulo della grandezza di un pisello interposto e saldante il lobo sinistro del fegato con la milza. In qualche caso zone circoscritte di parenchima pancreatico appaiono chiaramente lese ed in preda a fenomeni necrotici. Il rene ed il fegato appaiono pallidi ed edematosi, la milza aumentata di volume con evidenziazione della polpa bianca; i polmoni congesti ed in parte enfisematosi, al taglio, presentano quache focolaio di addensamento parenchimale; il cervello mostra modeste note di iperemia al livello delle meningi.

Nei ratti inoculati endovena il reperto più saliente si riscontra a livello dei polmoni che mostrano aree di colorito rosso cupo, di consistenza aumentata alternate a zone tipicamente enfisematose. Sia all'esame esterno che al taglio, il parenchima polmonare appare sede di numerosi noduletti di grandezza variabile da una capocchia di spillo ad un grano di miglio ed anche più, a volte confluenti, di colorito bianco giallastro; tali noduli appaiono più evidenti in sede sottopleurica.

Nei due ratti inoculati nel testicolo e sacrificati dopo 14 giorni si notano, nel testicolo inoculato, una o più masserelle della grandezza di un grano di riso ad un pisello, di colorito bianco giallastro, di aspetto necrotico.

Nel gruppo di ratti inoculati per via intracranica, sia negli animali morti spontaneamente che in quelli sacrificati, il reperto più saliente e costante, all'apertura della scatola cranica, è rappresentato da una diffusa iperemia meningea. Al taglio dell'encefalo abbiamo riscontrato circoscritti focolai di emorragia e di necrosi e piccolo focolai ascessuali nel parenchima nervoso e specialmente a livello delle cavità e del rivestimento ependimale dei ventricoli. Il ratto sacrificato in settima giornata, in corrispondenza della teca interna ed in sede di inoculazione, presentava numerose piccole formazioni nodulari della grandenzza di una capocchia di spillo ed anche più, di colorito grigio bruno-rossastro estendentisi alla vicina zona temporale. Nei ratti sacrificati in quarta e settima giornata un reperto degno di nota si nota a carico dei polmoni. Tali organi, sopratutto in sede subpleurica, presentano una disseminazione di noduletti di varia grandezza da una capocchia di spillo a un grano di miglio ed anche più, isolati o confluenti, di colorito bianco giallastro.

Reperti microscopici.

L'osservazione istologica dei numerosi preparati allestiti su vari organi dall'aspetto macroscopico apparentemente indenne o già decisamente alterato, permette di ricostruire il ciclo completo rappresentante il graduale evolversi delle lesioni. E'degno di particolare nota il fatto che, dovunque l'Actinomicete in esperimento abbia preso impianto, il processo evolve con modalità perfettamente analoghe e costanti.

Si ha dapprima una diffusa presenza di elementi fungini, elettivamente evidenziabili con le specifiche colorazioni, sparsi, senza particolare strutturazione architettonica, fra gli elementi costitutivi del parenchima colpito. Ben presto l'organismo infettato mostra di accorgersi della presenza del parassita e già nelle prime giornate dopo l'inoculazione si manifesta una reazione flogistica sostenuta da elementi parvicellulari, prevalentemente rappresentati da leucociti neutrofili, che accorrono in gran copia e costituiscono imponenti infiltrati con aspetti similascessuali. Il tessuto ospite mostra nel contempo più o meno prominenti segni di sofferenza parenchimali con necrosi di gruppi cellulari o di intere porzioni d'organo. Vediamo a questo stadio manifestarsi quadri di broncopolmonite, di pancreatite, di orchite, di meningo-encefalite, a seconda delle vie di inoculazione. Il pancreas è leso nella sua componente esocrina in vari lobuli; qualora, oltre alla flogosi acuta, si arrivi alla necrosi acinosa, anche le isole del territorio colpito scompaiono. Nel testicolo, tra le porzioni centrali di orchite necrotica ed il parenchima indenne alla periferia, esiste una fascia di transizione in cui si apprezzano tubuli seminiferi più o meno zaffati di elementi fungini e con cellule seminali e sertoliane profondamente alterate. Vi compaiono voluminose cellule giganti sinciziali, soggiacenti a processi regressivi simili a quelli presenti in banali orchiti sperimentali od in ratti sottoposti a particolari trattamenti ormonici (MARTINAZZI, 1960). In un animale (R/3) inoculato per via endoperitoneale il già riferito reperto macroscopico di congestione meningea si è rivelato invece come processo meningitico acuto con diffusa presenza di polimorfonucleati neutrofili; le colorazioni specifiche non hanno pur tuttavia evidenziato elementi fungini.

La successiva evoluzione della forma micopatologica denuncia il solitamente massivo intervento di cellule istiocitarie, che subentrano, rimpiazzandoli completamente, agli ammassi di elementi parvicellulari. Questi istiociti si organizzano ben presto a circondare, con disposizione a raggiera od a palizzata, le colonie fungine costituite da tenui filamenti variamente intrecciati a formare sia un feltro sia un cespuglio con disposizione degli elementi filamentosi periferici atteggiantisi a tipica "drusa" actinomicotica. Alla periferia di queste druse fungine, circondate da corona istiocitarie ad elementi cilindrico-allungati, molte altre cellule istiocitarie si dispongono con distribuzione casuale, accompagnandosi a scarsi elementi granulo-linfocitari ed a più frequenti fibroblasti, tanto da costituire ben individualizzabili "granulomi fungini". Non è infrequente vedere come nel contesto del granuloma compaiano qua e là elementi gigantocellulari, plurinucleati, esplicanti spiccata attività macrofagica. Le colorazioni col Gram o col PAS dimostrano infatti, nei voluminosi citoplasmi di queste cellule giganti, abbondanti residui fungini, mentre il citoplasma di interi nidi istiocitari dimostra intensa PAS-positività. Gli elementi fungini vengono cosi

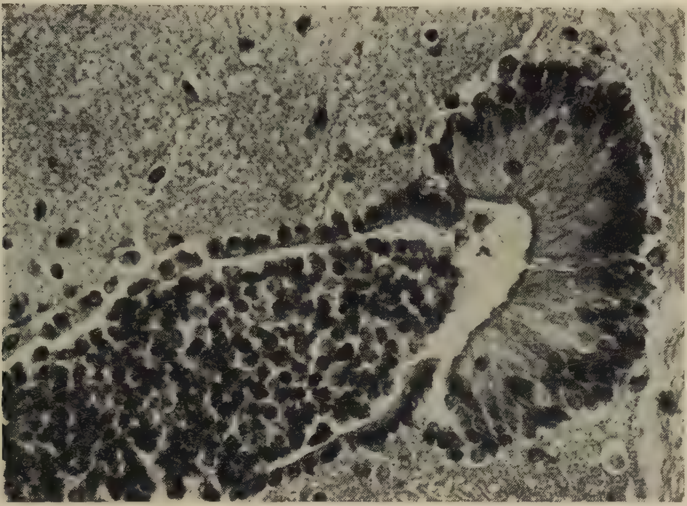

Fig. 1. Ratto/30; inoculazione endocranica; morto ad 1 giorno.
Cervello: nella cavità del terzo ventricolo è raccolto abbondante essudato polimorfonucleato, in cui la colorazione di Gram mette in evidenza numerosi filamenti fungini. Em.-eos. × 500.

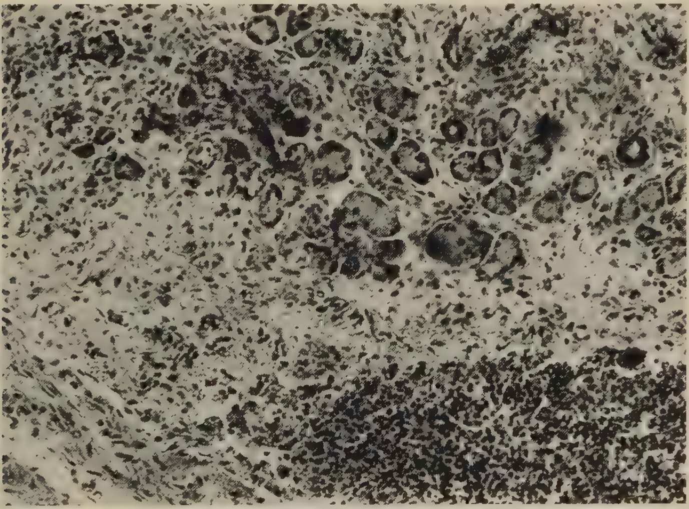

Fig. 2. Ratto/4; inoculazione endoperit. morto a 6 giorni.
Pancreas: il parenchima pancreatico è gravemente sofferente ed appare infiltrato da polimorfonucleati con elementi parvicellulari e parzialmente sostituito da giovane tessuto connettivale. Em.-eos.; × 160.

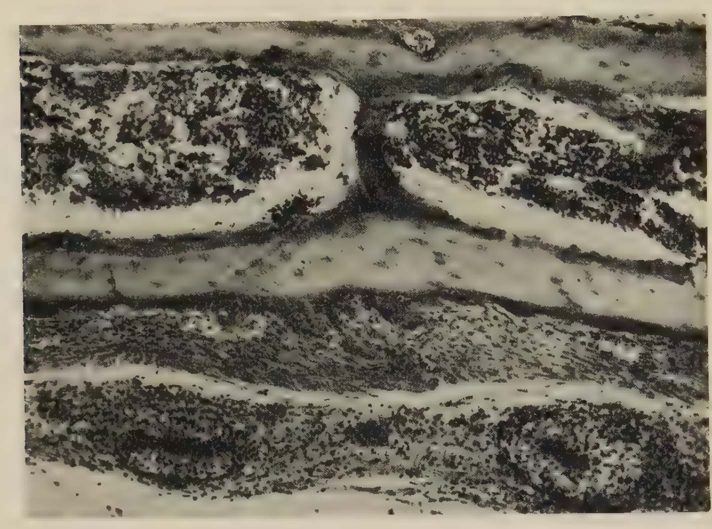

Fig. 3. Ratto/28; inoculaz. endocranica; ucciso a 7 giorni.
Teca cranica: sono bene apprezzabili i due tavolati ossei con interposizione di midollo normale, mentre all'interno della vitrea la pachimeninge mostra un patologico ispessimento e segni di flogosi. Più profondamente uno strato fibro-edematoso contiene cellule infiammatorie, istiociti e due caratteristiche "druse" dimostranti tipica architettura con colonia fungina al centro e corona radiata istiocitaria. Em.-eos.; × 140.

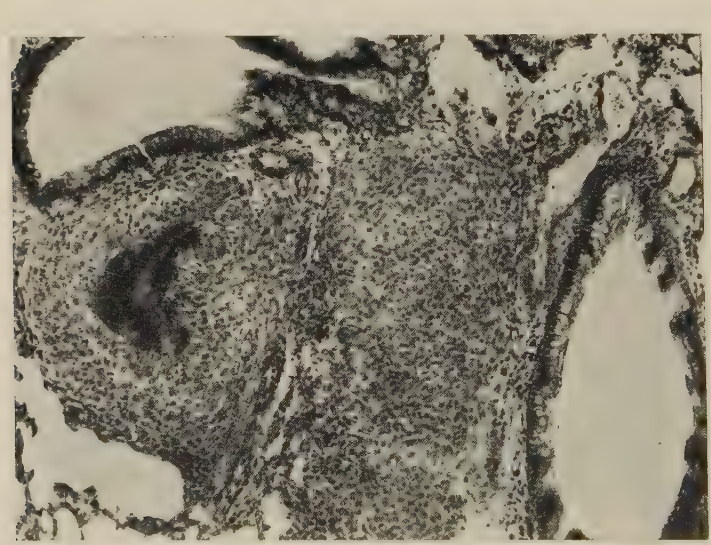

Fig. 4. Ratto/22; inoculaz. endovena; ucciso a 10 giorni.
Polmone: granulomi fungini peribronchiali con quadri di polmonite interstiziale. Em.-eos.; × 150.

ad essere progressivamente demoliti e rimossi dal focolaio di infezione e possono in certo qual modo venir seguiti, nella loro graduale sofferenza, mediante l'indagine abbinata con le colorazioni di Gram e di Hotchkiss. Vediamo infatti che mano a mano che il fungo viene sempre più validamente attaccato dalle difese tissulari esso perde dapprima la sua Gram-positività per diventare poi, quando è ormai ridotto ad un ammasso di elementi coccioidi, anche PAS-negativo.

Alla suddescritta fase granulomatoso-produttiva subentra, con vario lasso di tempo, una proggressiva maturazione di fibroblasti in fibrociti, una graduale organizzazione di tralci collageni ed una scomparsa sia degli istiociti che delle forme giganto cellulari. Il primitivo focolaio di infezione risulta pertanto deterso sia dall'agente flogogeno, che dalla componente reattivo infiammatoria, che infine, dai prodotti di demolizione necrotica dei tessuti primitivamente colpiti. Residuano pertanto, come esito tardivo della superata infezione, banali centri sclero-cicatriziali.

L'esame istologico del fegato e dei reni, già macroscopicamente denuncianti anormalità, ha costantemente permesso di apprezzare una sofferenza parenchimale di tipo metamorfosico vacuolare e torbido del tutto aspecifica ed imputabile alla generica presenza di tossine esogene ed endogene, comune a tutti i processi infettivi. Anche nella voluminosa milza di tutti gli animali inoculati sono sempre stati rilevati i quadri di una intensa congestione con attivazione della polpa rossa ed iperplasia della polpa bianca, quadri caratteristici della splenomegalia spodogena.

Il sistematico esame istologico delle linfoghiandole satelliti degli organi colpiti non ha mai rivelato interessamento micotico di tali disdretti, ma soltanto note di linfoadenite reattiva aspecifica.

DISCUSSIONE

Circa gli aspetti microscopici del fungo nelle lesioni, una prima osservazione che ci sembra di un certo interesse riguarda la assenza nell'*A. philippinensis* animalizzato, di zoosporangi e di zoospore. Nelle lesioni l'Actinomicete si presenta per lo più sotto forma di ife sottili, delicate di 0,5-1μ di diametro, diritte od ondulate, più o meno ramificate le quali possono form are deifeltri variamente intrecciati o assumere aspetti di veri cespugli con disposizione periferica a raggiera fino a ricordare la tipica "drusa" actinomicotica (il che deve fare pensare ad un progressivo adattamento del patogeno all'ambiante e ad una sua colonizzazione con tendenza invasiva) o a volte invece frantumarsi in piccoli elementi coccoidi o bacillari come avviene, sia su mezzi culturali artificiali sia nella vita parassitaria per altri Actinomycetales dei generi *Nocardia* ed *Actinomyces*, ed anche per lo stesso *Actinoplanes*, dopo essere stato coltivato a lungo su terreni artificiali. Nel corso delle molte osservazioni da noi compiuti non ci è stato possibile infatti rilevare nei tessuti nessun elemento riconducibile od equivalente degli sporangi da noi osservati nei preparati a fresco delle colture di *A. philippinensis*. La mancata formazione di sporangi e quindi di spore mobili può essere spiegata: a) con le mutate condizioni ambientali in cui l' *Actinoplanes* si trova crescere nella vita parassitaria. In natura l' *A. philippinensis* o le altre specie di *Actinoplanes* formano più facilmento sporangi su foglie di erba in acqua (l'acqua sembra essere elemento indispensabile per il nuotare libero delle zoospore quando escono dallo sporangio). Possono anche formarsi, però, meno constantemente e con maggiore difficoltà, su terreni artificiali (Czapek agar, Krainsky's agar etc.). b) col fattore tempo: mentre in natura su foglie in acqua la formazione di sporangi può avvenire entro dieci giorni, su terreni ertificiali, per ottenere tale formazione occorrono da sei-nove settimane. E'evidente che, in condizioni di vita parassitaria, le difese istiali dell'organismo ospite, intervengono molto prima che questi elementi di riproduzione abbiano avuto modo di svilupparsi. Per l'assenza di zoosporangi e di zoospore l'*A. philippinensis*, nei tessuti del ratto viene quindi ad assumere una morfologia che ricorda quella di alcuni actinomiceti patogeni, sia con formazioni raggiate rievocanti la"drusa" actinomicotica, sia con forme coccioidi o bacillari isolate o in ammassi. Pure interessante e costante è stata l'osservazione delle modificazioni morfologiche, da interpretarsi quali espressioni della sofferenza cui va incontro il micete, mano a mano che si procede nel tempo rispetto alla data d'inoculazione e con l'intensificarsi ed il prevalere delle complesse reazioni umorali ed istiali di difesa. Tale stato di sofferenza del fungo si manifesta sia con la netta diminuzione delle colonie atteggiantesi a "drusa" e con un frammentarsi degli esili filamenti in elementi coccoidi e bacillari che appaiono numerosi sia fuori, che in posizione endocitoplasmatica negli istiociti e nelle eventuali cellule giganti, ma anche e sopratutto con una modificazione dei suoi caratteri tintoriali. Più volte abbiamo potuto osservare infatti come l'Actinomicete, mentre viene sopraffatto dagli elementi di difesa, sembri perdere gradualmente la sua affinità per il Gram pur conservando la possibilità di colorarsi intensamente con il metodo di Hotchkiss. In taluni granulomi i tenui filamenti ci sono apparsi infatti o totalmente o in parte Gram-negativi e per contro PAS positivi come se il micete, in preda a processi disintegrativi, andasse dapprima incontro ad una graduale perdita della Gram positività pur conservando la PAS positività legata a frazioni polisaccaridiche delle sue pareti cellulari.

Per quanto riguarda invece i dati anatomo-patologici dobbiamo prima di tutto sottolineare che un 'azione patogena dell' *A. philippinensis* è stata rilevata, ma che, in complesso, esso possiede scarsa aggressività ed invasività. Ne fanno fede la limitazione delle lesioni alle sedi di inoculazione (pur con infrequenti possibilità di colonizzazione ematogena a distanza) nonchè la relativa facilità con cui gli organismi degli animali infettati riescono a far fronte ed a superare la micosi. Ciò non toglie che all'incirca un

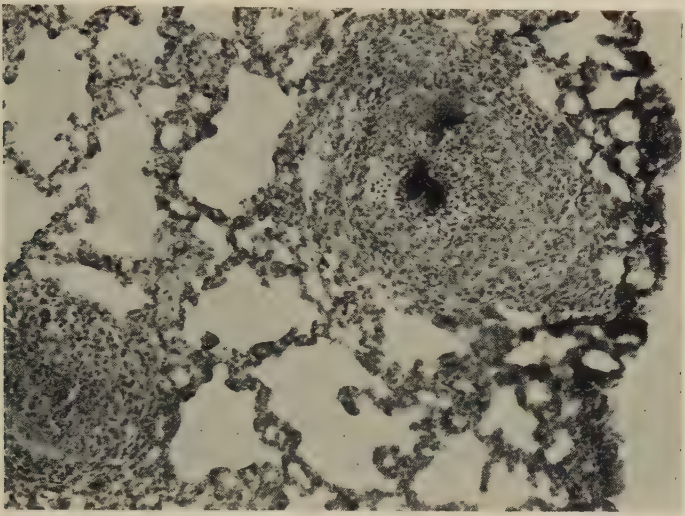

Fig. 5. Ratto/22; inoculaz. endovena; ucciso a 10 giorni.
Polmone: granulomi fungini in sede sottopleurica con reazione istio-gigantocellulare ed irregolari ispessimenti settali ad opera di comuni cellule flogistiche. Em.-eos.; × 140.

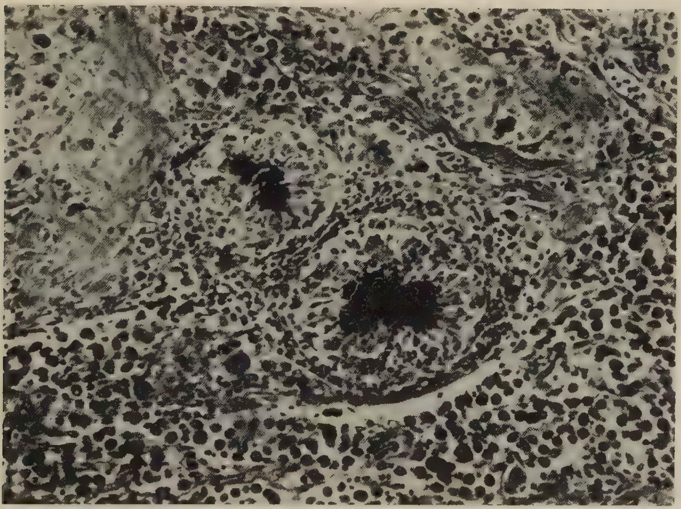

Fig. 6. Ratto/10; inoculaz. endoperit.; ucciso a 14 giorni.
Tessuti connettivo adiposi peripancreatici: tipici granulomi con cellule istiocitarie allungate e disposte a raggera attorno alle "druse" centrali. Nei connettivi lassi circostanti sono bene apprezzabili elementi flogistici parvicellulari e numerosi istiociti intensamente PAS-positivi. La presenza di alcuni tralci connettivali denota iniziale evoluzione sclerotica del focolaio. PAS.; × 180.

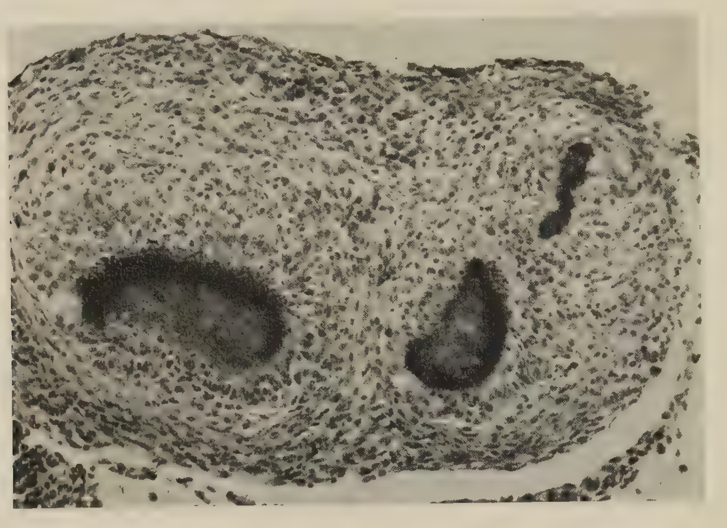

Fig. 7. Ratto/17; inoculaz. endoperit.; ucciso a 12 giorni.
Tessuti connettivo-adiposi peripancreatici: granulomi agminati in sede sottoperitoneale denunzianti tipiche "druse" fungine a periferica iniziale organizzazione connettivale. Em.-eos.; × 180.

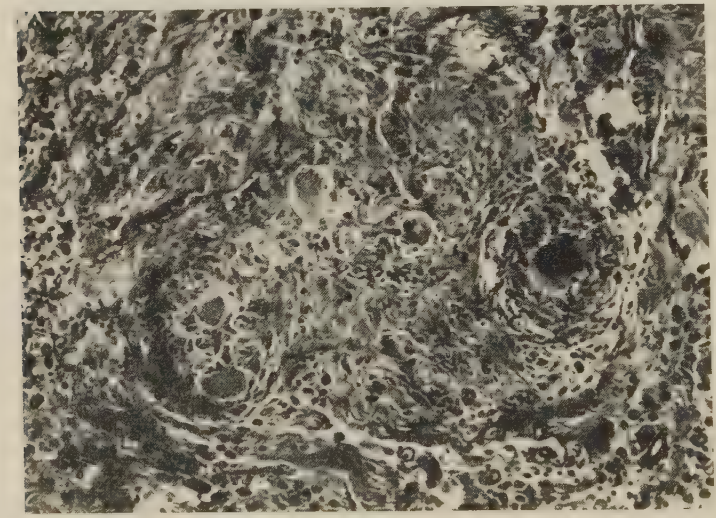

Fig. 8. Ratto/9; inoculaz. endoperit.; ucciso a 14 giorni.
Tessuti connettivo adiposi peripancreatici: granuloma con residui elementi fungini, polimorfonucleati, cellule epitelioidi, cellule giganti in attività fagocitaria ed avanzati processi sclerotici. PAS; × 180.

animale su tre venga ucciso dall'infezione prima che le sue difese abbiano potuto adeguatamente porsi in attività.

Il risentimento generale dell'organismo è pur sempre evidente come di fronte a banali insulti flogistici il che pare avvalorato dalla costante sofferenza denunciata da fegato, rene, e milza. La reazione istochimica con il PAS ha evidenziato un quadro di particolare interesse sulla cui interpretazione non è possibile che avanzare una ipotesi. Laddove ai processi flogistico-essudativo si va sostituendo la reazione istiocitario-granulomatosa, si apprezza come le cellule istiocitarie, disposte a palizzata attorno alla colonia fungina, siano di norma PAS negative, presentando soltando eventuali frammenti fungini inclusi come reagenti positivamente allo Schiff. Gli istiociti invece dislocati tutt'attorno, hanno il citoplasma intensamente ed omogeneamente PAS positivo e presentano forma sferoidale con diametri alquanto variabili. Ci sembra quindi verosimile pensare che ciò sia espressione di qualcuno dei complessi meccanismi bioumorali, collegati all'infiammazione, e nel presente tipo di infiammazione micotica, abbondantemente agganciati al biochimismo delle sostanze mucopolisaccaridiche.

Nonostante la sua modesta aggresività ed invasività, ci sembra poter senz'altro affermare che l'*A. philippinensis*, alla luce di questi primi dati sperimentali, è un patogeno potenziale per gli animali e per l'uomo data la possibilità di inoculo da substrato di vita ex natura, attraverso spine, ferite etc.

Non avendo eseguito prove circa un'eventuale patogenicità dell'*A. philippinensis* per gli animali da laboratorio per vie diverse da quella parentereale, non abbiamo, per ora, elementi per discutere intorno ad una possibile epidemiologia in natura; non abbiamo neppure elementi per affermare una patogenicità sperimentale di questo peculiare actinomicete su animali diversi dal ratto, pur notando essere questo animale relativamente poco suscettibile alle infezioni fungine sperimentali. Prove successive potranno chiarire anche questo aspetto di una potenziale epidemiologia.

Ci sembra comunque notevole e di particolare interesse il fatto che le culture di *A. philippinensis*, isolate da mezzi di vita soprafitica e dopo quasi tre anni di vita continuativa su substrati di laboratorio, abbiano mantenuto la loro patogenicità, cio che sta a significare non essere questo un fatto di adattamento temporaneo a particolari condizioni (a noi ignote) preculturali, ma un fatto intrinsecamente legato alla fisiologia del fungo, e quindi costantemente connesso con le sue attività metaboliche. Ciò è confermato anche dall'aspetto che il micete assume in certi tessuti e che si riconnette ai quadri usuali anatomopatologici provocati dagli actinomiceti patogeni (e più particolarmente a quelli della *Nocardia asteroides*); da ciò consegue anche che il tipo generale della reazione istiogena si riporta a quello generale prodotto dagli Actinomiceti.

Il fatto che l'*A. philippinensis* sia rimasto patogeno dopo vari anni ci cultura, sembrerebbe deporre, in un certo senso, a favore di una somiglianza di comportamento con i funghi forse più che con gli actinomiceti dei genere *Nocardia* ed *Actinomyces*, i quali già non facili a provocare lesioni patologiche da culture di primo isolamento o da materiale biologico, perdono rapidamente in successive subculture su terreni usuali di laboratorio ed a temperatura ambiente, la loro patogenicità, quando, almeno certi gruppi di funghi inferiori, sembrano conservarla meglio.

Tabella I.

Osservazioni sperimentali su ratti inoculati con Actinoplanes philippinensis (*Couch*).

Animale N.	Peso corporeo g.			Via di inoculazione	cc. inoculati	Esito: + = morto ° = ucciso a giorni
	iniziale	a 4 giorni	finale			
R/11	148		140	Endoperitoneale	1	+ 2
R/3	120		115		1	+ 3
R/13	135		120		1	+ 3
R/14	146		110		1	+ 5
R/4	130		120		1	+ 6
R/12	143		105		1	° 8
R/15	130		121		0,7	° 8
R/17	130		145		0,7	° 12
R/9	160		160		1	° 14
R/10	166		167		1	° 14
R/16	135		150		1	° 15
R/18	145		205		1	° 40
R/19	136	120	137	Endovenosa	0,5	° 7
R/21	140	147	157		0,5	° 10
R/22	135	120	140		0,8	° 10
R/23	145	120	140		0,8	° 13
R/24	132	137	150		0,8	° 13
R/7	160		150	Endotesticolare	0,7	° 14
R/8	160		165		0,7	° 14
R/30	150		140	Intracranica	0,3	+ 1
R/27	180		160		0,5	+ 1½
R/25	157		115		0,5	° 4
R/28	160		165		0,5	° 7

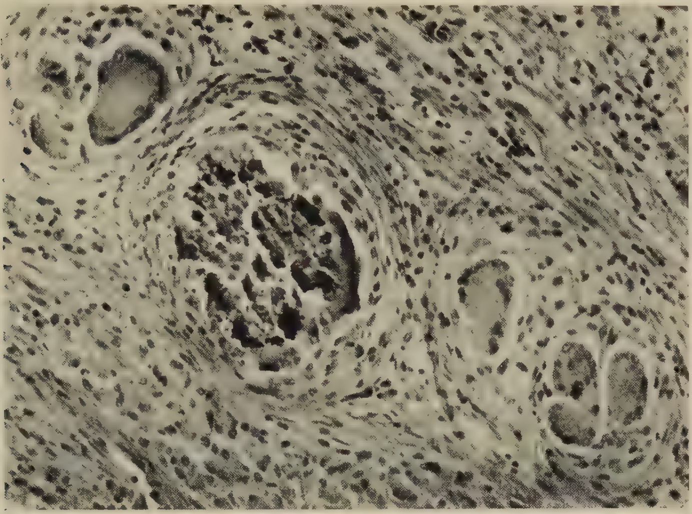

Fig. 9. Ratto/8; inoculaz. endotesticolare; ucciso a 14 giorni. Testicolo: con granuloma fungino, cellule giganti, in attività fagocitaria e diffusa sostituzione sclerotica dei tubuli seminiferi primitavamente necrosati. Em.-eos.; × 200.

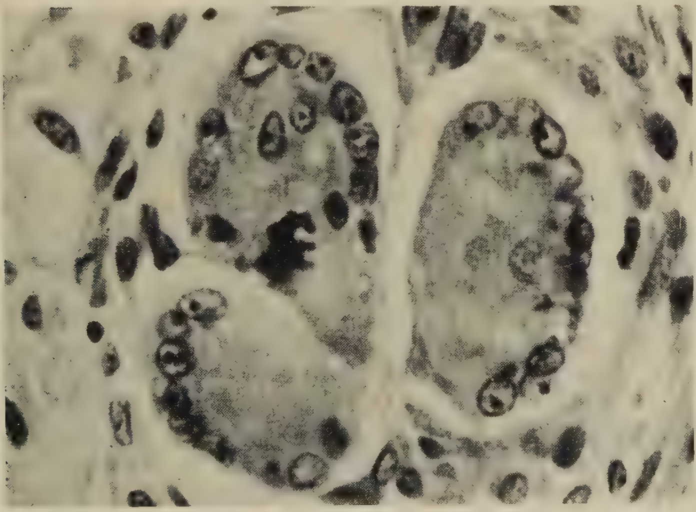

Fig. 10. Particolare della fig. 9. Sono bene apprezzabili voluminose cellule giganti con residui fungini fagocitati ed in via di demolizione. Em.-eos.; × 900.

SOMMARIO

SULLA PATOLOGIA SPERIMENTALE DA *ACTINOPLANES PHILIPPINENSIS* COUCH

Dopo una breve descrizione delle caratteristiche culturali e morfologiche dell'*A. philippinensis*, si riferiscono i risultati sperimentali ottenuti inoculandolo per via endoperitoneale, endovenosa, endotesticolare ed endocranica in ratti albini maschi del peso medio di 150 g. Morte spontanea si è verificata in circa un animale su tre.

I principali reperti sono: elettivo interessamento di determinati organi in rapporto alle varie vie d'inoculazione: peritoneo, pancreas e tessuti peripancreatici (nell'inoculazione endoperitoneale), polmone (nell'inoculazione endovenosa), testicolo (nell'inoculazione endotesticolare), encefalo (nell'inoculazione endocranica), mentre, indifferentemente nei diversi gruppi di animali, appaiono interessati da processi patosici aspecifici fegato, milza e reni. Ovunque abbia preso impianto l'actinomicete allo studio, il processo evolve, con modalità perfettamente analoghe e costanti, da quadri iniziali di flogosi acuta, con possibile componente necrotica del parenchima invaso, verso il quadro tipico del "granuloma fungino produttivo", con esito tardivo sclero-cicatriziale.

L'*A. philippinensis* animalizzato è caratterizzato: dall'assenza di zoosporangi o di elementi ad essi equivalenti; dimostra una discreta patogenicità nei confronti del ratto albino, con quadri istologici simili a quelli provocati da altri Actinomiceti patogeni, ed una scarsa tendenza invasiva e diffusiva.

SUMMARY

EXPERIMENTAL PATHOLOGY BY *Actinoplanes philippinensis* COUCH

The morphological and cultural characters of *A. philippinensis* are described, and experimental results of intraperitoneal, intravenous, intratesticular and intracranial inoculations on male rats are referred to. One out of three animals died spontaneously. The outstanding results are: some organs are selectively damaged by the lesions, viz: peritoneum, pancreas, peripancreatic tissues (after i.p. inoculation), lungs (after i.v. inoculation), testiculi (after intratesticular inoculation), brain (after intracranial inoculation), while in all animals the liver, spleen and kidneys show some signs of aspecific lesions. Wherever the *Actinoplanes* is present and grows the pathologic processes develop in the same way, with a constant and uniform pattern, from initial acute inflammation, with possible necrosis of the parenchyma, into typical "productive fungous granuloma" leading to fibrosis.

A. philippinensis in tissues is characterized by lack of zoosporangia

or their equivalent, by some degree of pathogenicity for the rat with histological pictures resembling those produced by other pathogen Actinomycetes and by its slight tendency to invasion and diffusion.

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HALLAZGO DE MASONIELLA GRISEA G. SMITH EN EL SUELO DE GALLINEROS Y COMPROBACIÓN DE SU PATOGENIDAD PARA ANIMALES DE LABORATORIO.[1]

por

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(1.XII.1960)

(con 6 figs.)

Después de haber encontrado casos humanos de histoplasmosis en autopsias (Salfelder & Capretti, 1959) y la prueba de histoplasmina positiva en un cierto porcentaje de la población en nuestro medio (Moncada, Sequera, & Gabaldón 1957) nos estamos dedicando en el momento actual a estudiar si existe *Histoplasma capsulatum* en el suelo de nuestras regiones y en cuales zonas se encuentra preferentemente.

Examinamos sobre todo muestras procedentes de gallineros en vista de que está comprobado que el hongo tiene su habitat con preferencia en éste medio.

En el curso de estos estudios cuyos resultados positivos se darán a conocer oportunamente, vimos crecer en los cultivos un micete poco conocido, cuyos carácteres patogénicos están desconocidos hasta la fecha y que fué clasificado como *Masoniella grisea* G. Smith.

La técnica empleada es la que utilizó inicialmente Emmons (1949) con las modificaciones descritas por Ajello y colaboradores (1960) y consiste en inocular por via intraperitoneal muestras preparadas de suelos a ratones. Hemos inoculado con cada muestra de tierra 8 ratones sacrificando 2 de ellos a las cuatro semanas, 4 a las seis, y 2 a las ocho semanas después de la inoculación respectivamente. Las autopsias de los animales se realizaron bajo cautelas estériles sembrando piezas de hígado y bazo en el medio de Sabouraud con antibióticos y actidione según Georg y col. (1954). Además de los cultivos se practicaron exámenes histopatológicos preferentemente de los hígados y bazos de los ratones.

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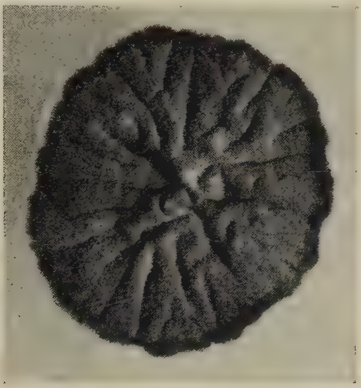
[1] Trabajo presentado en forma abreviada en el I. Congreso Panamericano de Biología y Patología Experimental, septiembre 1960, Caracas/Venezuela.

RESULTADOS DE LOS ESTUDIOS MICOLÓGICOS

La especie *Masoniella grisea* creció en 32 muestras, representando éste número el 26,4% de la muestras de suelo inoculadas a los ratones, habiendose estudiado hasta la fecha de la publicación de éstas lineas un total de 121 muestras.

Resultaron positivos un número total de 52 ratones inoculados con un número de 64 cultivos. En varios casos, *M. grisea* creció en los cultivos junto con *Histoplasma capsulatum.*

Considerabamos el hongo al principio como ,,contaminante de laboratorio'', sin embargo, en vista de que el hallazgo se repitió especialmente en material procedente de animales distintos pero inoculados con la misma muestra de tierra, llegamos a la conclusión que el hongo vivía en la tierra misma.



Fotogr. 1. Colonia de *Masoniella grisea* a los 20 dias en medio de *Sabouraud* glucosa. Tamaño natural.

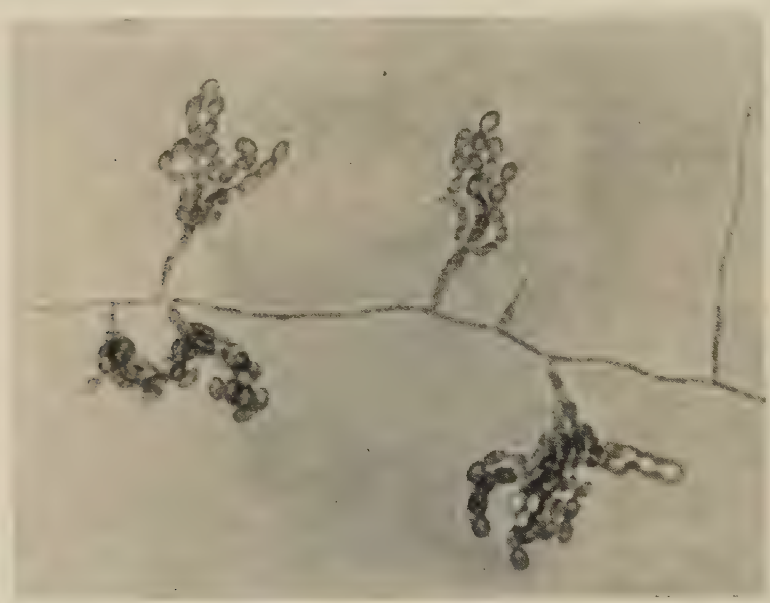
Las colonias se desarrollan lentamente, tienen un aspecto aterciopelado de color gris ceniza y una superficie arrugada con surcos radiales (ver fotografía N° 1). Con el tiempo el color gris se transforma en verdoso oscuro y llega a ser casi negro. El revés de la colonia se presenta de color gris-oscuro desde el comienzo del desarrollo, pero el pigmento no se difunde por el medio.

El crecimiento a la temperatura del laboratorio (24—26° C) es más rápido que en temperaturas más altas (37° C).

A los 10 dias de la siembra en medio de SABOURAUD glucosado, las colonias a la temperatura del laboratorio alcanzan 1,5 cms. de diámetro, mientras que el crecimiento apenas se aprecia a la temperatura de 37° C.

Sembrado en gelatina sí produce una lenta licuación.

La morfología microscópica es la siguiente: el micelio de color verde-aceituna, muy palido, tabicado y ramificado, con un diámetro variable entre 1,5—4 micras. Se realiza una pronta y abundante producción de estructuras fértiles representadas por fialides sésiles y aisladas o muy a menudo agrupadas en verticilos de aspecto peniciloide que llevan cadenas largas de conidios color verde aceituna, globosos, elípticos o más bien con formas características de ,,tonel'' cuando aún no se han desarticulado de las cadenas (ver fotografía N° 2).



Fotogr. 2. Estructuras fértiles de *M. grisea*: hifas conidioforas, fialides, cadenas de conidios.

Los conidios són unicelulares, de pared lisa y miden 2,5—4 × 4—5 micras de diámetro menor y mayor respectivamente. Las fialides se presentan ensanchadas en sus partes centrales y miden 2,5—3 × 5—8 micras.

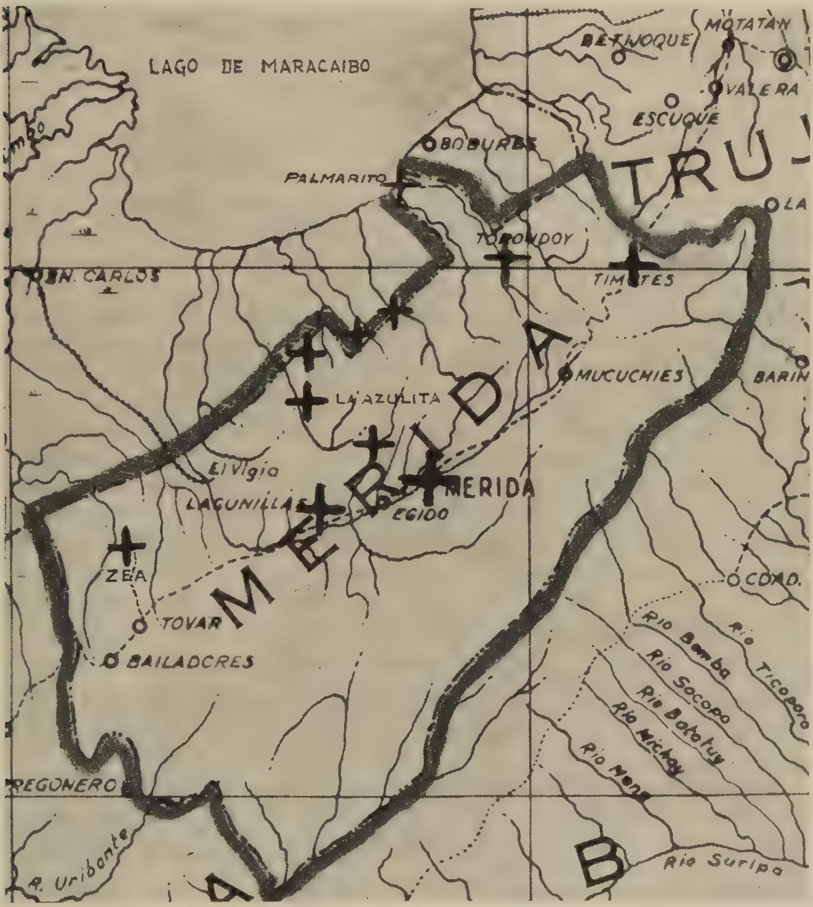
Cuando las fialides se encuentran agrupadas en verticilos, la hifa conidiófora de la cual se origina el ,,pincel'' puede ser corta, sin embargo alcanza a veces una longitud de 20 micras desde la base hasta la primera ramificación.

Proveniencia de las muestras positivas y apuntes geográficos

Las muestras de tierra positivas provenian de los lugares siguientes: Ciudad de Mérida 7, La Azulita 6, Palmarito 3, Caño

Zancudo y El Valle grande (Mérida) 2, Aldea Chama (Mérida), Agua Blanca, Agua Azul, Arapuey, Chorros de Milla (Mérida), Lagunillas, Las González, La Otra Banda (Mérida), Mesa de Ejido, San Cristóbal de Torondoy, Timotes y Zea: 1.

Es decir, el hongo vive en alturas sobre el nivel del mar desde 0 a 2000 mts. y en tierras de muy diferentes clases (ver fotografía N° 3, mapa del Edo. Mérida/Venezuela y de sus Estados vecinos).


LAGO DE MARACAIBO
BETIJOQUE
MOTATAN
VALERA
ESCUQUE
BOBURES
PALMARITO
TRU
LA
TORONDOY
TIMOTES
SN. CARLOS
MUCUCHIES
BARIN
LA AZULITA
MERIDA
El Vigia
LAGUNILLAS
MERIDA
EGIDO
ZEA
TOVAR
BAILADORES
CORD.
Rio Bomba
Rio Ticoporo
Rio Socopo
Rio Batatuy
Rio Michay
Rio Mena
REGONERO
R. Uribante
B
Rio Suripa

Fotogr. 3. Mapa del Edo. Mérida/Venezuela con los lugares (+) de los cuales provenian las muestras positivas para *Masoniella grisea*.

El Estado Mérida está situado en los Andes Venezolanos, al sur del Lago de Maracaibo en la situación geográfica siguiente: latitud entre 7° 47' norte y 9° 11' norte y longitud entre 70° 33' y 71° 80' W Gr.

Sus condiciones geográficas y su clima són muy variados puesto que el Estado comprende zonas netamente tropicales a la orilla del Lago y en sus extensos valles y otras enteramente andinas con montañas hasta 5000 mts. de altura. En éstas últimas se encuentran poblaciones hasta más allá de 4000 mts. de altura.

LESIONES ANATOMOPATOLÓGICAS EN LOS RATONES INOCULADOS CON MUESTRAS DE TIERRA.

De los 52 ratones en los cuales los cultivos de *Masoniella grisea* resultaron positivos 17, es decir casi la tercera parte demostraron lesiones en el hígado.

Se encontraron:

a) Infiltraciones celulares circunscritas preferentemente perivasculares. 11 veces
b) Granulomas submiliares con histiocitos, eosinófilos, discreto número de células gigantes y centros necróticos. 4 veces
c) Granuloma pequeño de cuerpos extraños. 1 vez.
d) Granuloma con hongos reconocibles. 1 vez.

Resumiendo puede decirse que la patogenidad del hongo proveniente del suelo directamente era muy baja, el hongo se encontraba una sola vez visible en un granuloma del tejido hepático habiendo dado lugar aparentemente a la formación del mismo.

Puede suponerse que el hongo há provocado también las demás lesiones mencionadas, sin embargo, al no encontrarlo a nivel de las lesiones tisulares mismas y en vista de que las infiltraciones celulares perivasculares (a) se vieron frecuentemente también en otros casos sin cultivo positivo, su posible acción patógena en estos casos es dudosa.

RESULTADOS DE INOCULACIONES CON EL CULTIVO DE MASONIELLA GRISEA

Quedó a estudiar la patogenidad de los cultivos de nuestro hongo, es decir sus carácteres patógenos después de haber vivido una vez en un animal.

Empleamos para nuestros ensayos 19 ratones realizando inoculaciones intraperitoneales e intracerebrales, 9 cobayos (acures) inoculados por via intraperitoneal, intracardiaca e intratesticular y 9 conejos practicando inoculaciones intraperitoneales, endovenosas e intracardíacas, sacrificando los animales en intervalos diferentes después de las inoculaciones.¹)

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¹) Las inoculaciones se realizaron con suspensiones bastante turbias en sol. salina esteril de cultivos en Sabouraud glucosa agarizado.

En los ratones por via intraperitoneal inoculamos 1 cc de la suspención, por via intracerebral una cantidad minima (una gota); en los cobayos por via intraperitoneal 2 cc, por via intracardiaca 1 cc, por via intratesticular 0,3 cc; en los conejos por via intraperitoneal 3 cc, por via endovenosa e intracardiaca 1 cc.

En los retrocultivos de diferentes órganos, preferentemente del hígado y del bazo se obtuvo en los ratones inoculados por via intraperitoneal cultivos positivos en todos los 14 animales empleados es decir, en un 100 % en intervalos desde 15 hasta 90 dias después de la inoculación, en piezas de hígado y bazo. Haciendo cultivos con liquido del lavado peritoneal obtenido durante la autopsia, comprobamos que los hongos se mantenian vivos en la cavidad abdominal hasta 30 dias después de la inoculación pero no más. También resultaron positivos todos los retrocultivos efectuados un mes despues de las inoculaciones intracerebrales.

En cuanto a los cobayos (acures) resultaron positivos los 3 retrocultivos del testiculo un mes después de la inoculación. Los retrocultivos de hígado, bazo, riñón, corazón de animales inoculados por via intraperitoneal e intracardíaca, resultaron negativos.

En los conejos quedó positivo un retrocultivo del bazo, 15 dias después de una inoculación por via endovenosa.

Resumiendo, se obtuvieron retrocultivos positivos en un alto porcentaje (100 %) en los ratones y también en un cierto porcentaje en los cobayos y conejos después de aplicaciones en distintos sitios, observando que el hongo aparentemente se acumula selectivamente en ciertos organos y permanece vivo en ellos hasta por 90 dias.

Anatómicamente, se encontraron lesiones granulomatosas conteniendo hongos, en los ratones y en los cobayos.

En los ratones, en 8 de 14 inoculados con cultivos intraperitonealmente se observaron granulomas preferentemente en el hígado, pero a veces también en el bazo o en la serosa del bazo y en el epiplón, con lapsos entre 14 y 90 dias después de la inoculación. Además se observó un pequeño granuloma con hongos en el encéfalo después de inoculación en éste sitio.

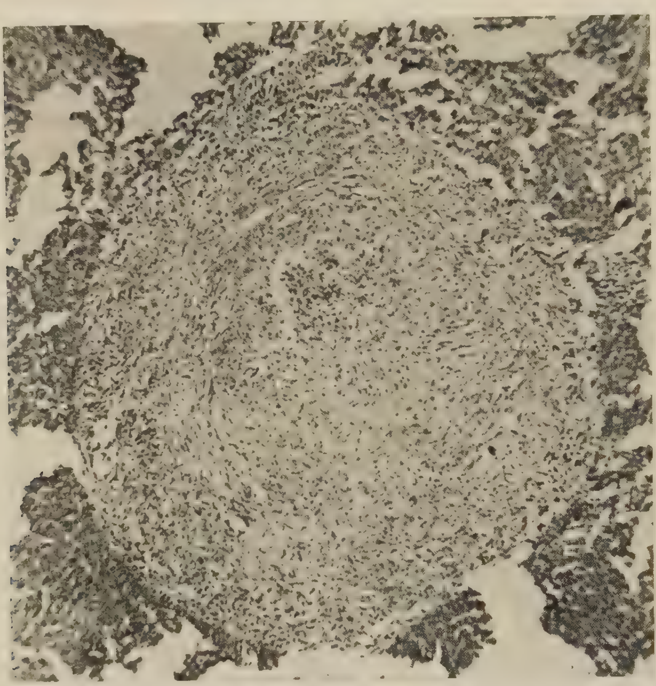
En cobayos se encontraron lesiones hepáticas también granulomatosas con hongos a partir de inoculaciones intraperitoneales y se hallaron un granuloma con hongos en los pulmones después de una inoculación intracardíaca y un granuloma con hongos a nivel del testículo después de una inoculación en el mismo sitio.

Desde el punto de vista morfológico, los granulomas frecuentemente eran macroscópicamente visibles de un tamaño menor que la cabeza de un alfiler, solitarios o múltiples.

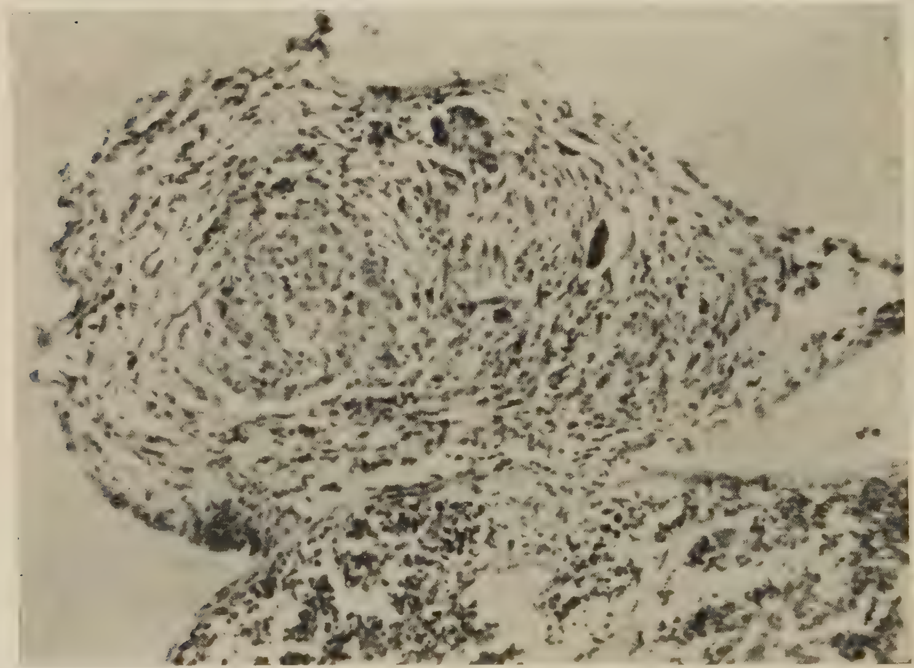
Microscópicamente los granulomas estaban constituídos por acumulaciones celulares; se encontraron granulocitos, a veces eosinófilos, histiocitos, células epitelioides y raras veces pocas células gigantes.

A nivel del centro se reconocieron a veces necrósis y en la periferia hacia el tejido vecino indemne, formación de tejido conjuntivo colágeno (ver fotografía N° 4 y 5).

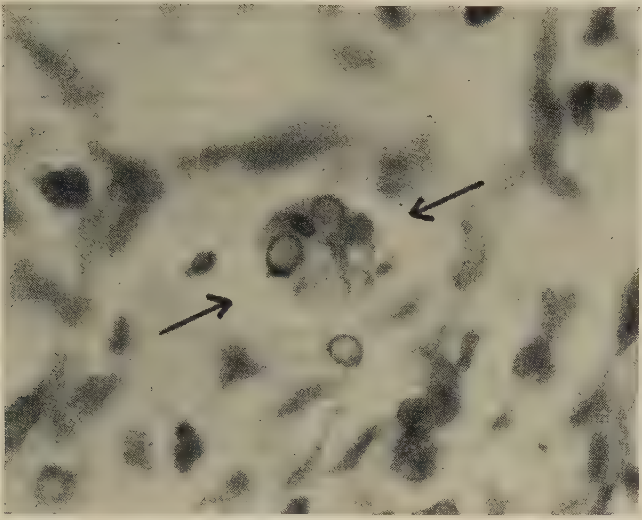
El micete a nivel de los granulomas lo hemos visto solo en la forma de conidios los cuales en su morfología eran parecidos a los que se ven en los cultivos.



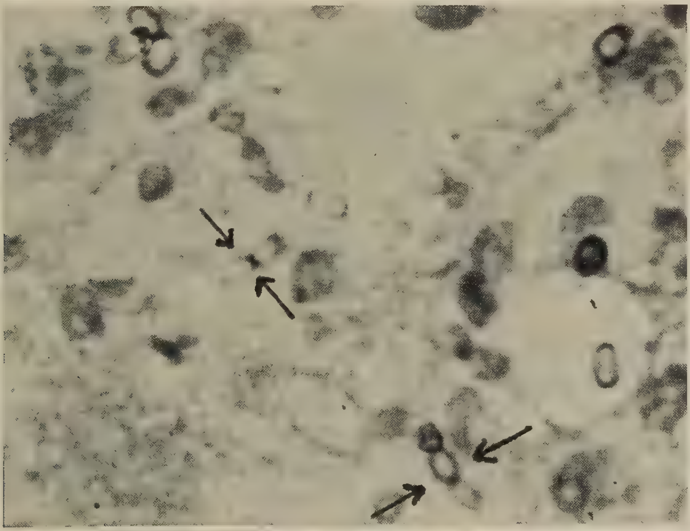
Fotogr. 4. E. No. 9866, ratón inoculado intraperitonealmente con cultivo de *Masoniella grisea* 3 semanas antes de ser sacrificado. Col. H. E. Aumento pequeño. Granuloma en el hígado (macroscópicamente granulomas submiliares).



Fotogr. 5. E. No. 10.887, ratón inoculado intraperitonealmente con cultivo de *Masoniella grisea*, 64 dias antes de ser sacrificado. Col. H. E. Aumento mediano. Granuloma pequeño con fibrosis en la serosa del bazo conteniendo hongos.



Fotogr. 6. E. No. 9866. Granuloma del hígado. Col. H. E. Aumento mayor. Formas conídicas de color amarillento en un cúmulo a nivel de una célula (↑).



Fotogr. 7. E. No. 9866. Granuloma del hígado. Col. H. E. Aumento mayor. Formas conídicas de color amarillento aisladas y en forma de cadenas (↑).

Estos conidios demostraron en H.E. un color amarillento y se tiñeron discretamente positivos en PAS y bien positivos en GMS. En cuanto a su disposición en los tejidos, se encontraron situados o en forma de acúmulos a nivel de una célula (ver fotografía N° 6), recordando en algo los hongos en la cromomicosis; o aislados o situados en forma de cadenas (ver fotografía N° 7).

Al resumir comprobamos un mayor número de lesiones granulomatosas conteniendo hongos después de la inoculación del cultivo del hongo que después de la inoculación directa de muestras de suelo; además resultaron susceptibles fuera de ratones también cobayos.

El hongo se asentaba y provocaba reacciones tisulares en hígado, bazo, epiplón y encéfalo de ratones y en hígado, pulmones y testículo de cobayos.

Las primeras lesiones se observaron 15 dias después de la inoculación; las lesiones más tardías con hongos presentes en los granulomas se debian a inoculaciones efectuadas hace 90 dias.

COMENTARIOS ACERCA DE LA CLASIFICACIÓN DEL HONGO Y CONCLUSIONES.

Logramos estudiar a un micete presente en el suelo de gallineros de nuestro ambiente desde la orilla del Lago de Maracaibo, hasta en sitios de 2000 mts. de altura en los Andes.

Lo considerabamos inicialmente — y de la misma manera de opinar eran algunos colegas — como perteneciente al gen. *Cladosporium.* Sin embargo, consultando DE VRIES (Centralbureau voor Schimmelcultures, Baarn, Holanda), experto universalmente reconocido del gen. *Cladosporium,* cuya colaboración agradecemos sumamente, el distinguido micólogo determinó la cepa como *Masoniella grisea* G. SMITH. Nosotros estamos de acuerdo con DE VRIES que los carácteres de nuestro hongo parecen corresponder a los de la especie antes nombrada.

El género *Masoniella* (Hyphales, Deuteromycetes), es poco conocido y fué descrito por G. SMITH inicialmente como *Masonia* (SMITH, 1952 a) y luego en el mismo año y por el mismo autor, como *Masoniella,* en vista de que el primer epíteto genérico ya había sido utilizado para denominar un ascomiceto (SMITH, 1952 b).

Del género *Masoniella* existen sólo dos especies: *M. grisea* y *M. chartarum.* La primera fué hallada como contaminante en una caja de PETRI en el laboratorio de la Escuela de Higiene y Medicina Tropical de Londres en el año de 1946; posteriormente no fué encontrada más. La segunda fué aislada del papel de tapicería de una habitación por el mismo SMITH en el año de 1950.

El hallazgo simple de la especie fungina *M. grisea* en nuestro ambiente sería ya por sí de cierto interés científico desde el punto de vista teórico,puesto que como lo apuntabamos arriba, aparentemente desde su primer hallazgo en el año 1946 en Inglaterra no existen otras publicaciones acerca de éste micete.¹)

Fuera de ésto sin embargo, el hongo en cuestión debe también interesarnos desde el punto de vista práctico, porque hemos podido comprobar su patogenidad en dos especies de animales, carácteres de él hasta ahora desconocidos.

Si bien el hongo provocaba a partir de la inoculación directa de muestras de tierra una sola vez un granuloma hepático, la inoculación del cultivo puro del mismo conducía a un número mucho más alto de reacciones tisulares.

Atribuimos esta discrepancia en parte a la cantidad de inoculum seguramente mayor utilizando suspensiones de cultivos que muestras de suelo y por otra parte puede suponerse que después del paso a través de los ratones el hongo haya aumentado su virulencia y patogenidad.

Terminamos nuestros comentarios recordando que ya varios autores realizando estudios exhaustivos en búsqueda de hongos patógenos para el hombre como *Histoplasma capsulatum* ,*Cryptococcus neoformans*, *Coccidioides immitis*, etc. en muestras de suelo, han encontrado otras especies patógenas generalmente poco conocidas (AJELLO, 1956; DOWING, 1947; EMMONS. y col., 1942; EMMONS, 1954; LURIE y col., 1955, y otros) pero no *Masoniella grisea*. EMMONS nos comunicó recientemente (1960) que muy a menudo él ha encontrado especies funginas en el peritoneo de los ratones las cuales sobreviven hasta un mes, pero sin producir lesiones anatómicas macroscópicas.

RESUMEN

1. En el curso del chequeo seriado de muestras de suelo de gallineros, recogidas en el Edo. Mérida/Venezuela y sus alrededores en búsqueda de *Histoplasma capsulatum*, se desarrolló en los cultivos de hígado y bazo de los ratones en más del 25 % de los casos un hongo perteneciente a la especie *Masoniella grisea* G. SMITH.

2. Se reportan los carácteres micológicos de la especie, su proveniencia con apuntes geográficos y se describen las lesiones anatómicas que produce el hongo en ratones después de inoculaciones directas de muestras de tierra y después de inoculaciones de cultivos del micete en ratones y cobayos.

3. Nuestro hallazgo de *Masoniella grisea* representa el segundo reporte de éste hongo en la literatura micológica después de la descripción de la especie por SMITH (1952).

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¹) Al redactar estas lineas nos comunica AJELLO (Head, Mycology Unit; Communicable Disease Center, PHS, Atlanta, Georgia, USA) quién estudió uno de nuestros cultivos de *Masoniella*, que durante sus investigaciones en búsqueda de *H. capsulatum*, há tenido la ocasión de ver cepas funginas parecidas sin haberlas determinado y sin haber comprobado sus caracteres patogenéticos.

Aprovechamos la oportunidad para expresar al Dr. AJELLO nuestro agradecimiento por su constante y sincera colaboración.

4. Mientras que la patogenidad de la cepa de SMITH no estaba conocida, la nuestra la demuestra claramente en ratones y cobayos.

SUMMARY

1. A fungus belonging to the species *Masoniella grisea* G. SMITH was found in subcultures of liver and spleen of more than 25 % of mice which had been inoculated with samples of soil from chicken yards. The samples had been collected in the state of Mérida (Venezuela) and vicinity in order to search for *Histoplasma capsulatum*.

2. This report describes mycological characteristics of the species *Masoniella grisea*, its distribution in the State of Mérida, with remarks on the geographic and climatic conditions of this area, and finally the anatomical lesions which became apparent after direct inoculation of soil into mice or after inoculation of the cultivated fungus into mice and guinea pigs.

3. As to our knowledge, this is the second report in the mycological literature on findings of *Masoniella grisea* after its first description by SMITH in 1952.

4. Whereas nothing is known about the pathogenicity of the strain G. SMITH, the strain described in this report exhibits definite pathogenicity for mice and guinea pigs.

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PREPARATION AND PROPERTIES OF THE ENDOTOXINS OF ASPERGILLUS FUMIGATUS AND ASPERGILLUS FLAVUS

by

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(with 6 figs.)

(12.XII.1960)

Introduction

Aspergillosis continues to be a problem in birds, especially in zoological gardens. The high incidence in captive penguins appears to be greatly reduced by rapid transportation under sanitary conditions from their normal habitat and maintenance in quarters equipped to furnish filtered air at low temperature. Success with this procedure suggests that the respiratory tract is an important route of infection. In domestic fowl ingestion of moldy feed has also been regarded as a source of infection (Asthana, 1944; Biester & Schwarte, 1952), but no experimental data are available, inasmuch as there has been little success in producing the characteristic slowly developing chronic disease by pulmonary or oral introduction of the spores. Nicolaus (1934) believed that a special susceptibility, such as induced by previous respiratory illnesses, was necessary for pathogenicity of the fungal spores. He investigated methods of intratracheal injection of hens with spores of *Aspergillus fumigatus*, suspended in fluids containing irritating substances, such as glass wool or alcohol. Even with suspensions in distilled water, he was able to induce small lesions which he studied histologically and regarded as characteristic minute aspergillus nodules. Herman & Sladen (1958) have reported inducing rapidly developing characteristic lesions in young chicks and in ducklings by direct inoculation of 10 to 50 million spores of *Aspergillus fumigatus* into a posterior thoracic air sac, but adult birds were able to survive inoculation with 500 million spores.

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[1] Deceased.

ELDER & BAKER (1956) found that spores of *Rhizopus arrhizus*, introduced intrabronchially into rabbits, induced fatal pulmonary mycosis if the animals were in the acute toxic stage of alloxan diabetes but not otherwise.

Extensive investigations of aspergillosis were carried out in France at the turn of the century (RENON, 1897; MACÉ, 1903). A fatal infection of laboratory animals, as well as of birds, was shown to be induced by intravenous injection of heavy suspensions of spores of *Aspergillus fumigatus*. Irregularities in the success of intravenous injection may well have been due to variations in numbers of spores introduced. In our experiments in rabbits (TILDEN, HATTON, FREEMAN & WILLIAMSON, 1957) we found that 10 million spores invariably induced fatal infection, while smaller doses might cause no obvious disturbance or might induce a generalized infection quite unlike the natural disease. We found also that heated spores were harmless but did not protect the rabbits against later injection of living spores, nor did a previous non-fatal infection protect the animals against a subsequent fatal dose.

The possibility that a toxin produced by *A. fumigatus* might be a significant factor in its ability to induce disease was investigated by several early French workers (LUCET, 1896; CENI & BESTA, 1902; MARTINS, 1928). BODIN & GAUTIER'S toxin (1906), which occurred in the culture medium after 2 to 3 weeks of incubation, was comparable to bacterial endotoxins in heat stability. It was, however, toxic to some species of animals resistant to *A. fumigatus* and not to the highly susceptible pigeon. Recently CARYL, FORGACS, HERRING & MABLANDT (1955) have reported that corn on which *A. fumigatus* had grown for 15 days was toxic for animals.

HENRICI (1939) described toxins which he obtained from young cultures of *A. fumigatus* and *A. flavus*. The mycelium was comminuted in a food grinder and the cell sap extracted by compression in a hydraulic press. The mycelial extracts, which were heat labile, were lethal for all species on which they were tested (rabbits, guinea pigs, mice and chickens). They were antigenic: rabbits receiving a series of gradually increasing doses became resistant to large doses. HENRICI reported one experiment which seemed to indicate that rabbits immunized against the toxin also acquired increased resistance to intravenous injection of spores of *A. fumigatus*. Subcutaneous injection of the toxic extracts into the rabbit skin induced severe lesions.

STANLEY (1950) separated acetone extracts of the mycelium of *A. fumigatus* into lipoid and polysaccharide fractions. The former induced tubercle-like lesions, the latter was antigenic, as shown by precipitin tests, but sera prepared with it were not protective against large doses of spores injected intravenously. He seems to have regarded the aqueous (saline) extract which he obtained by grinding the mycelium with sand as similar to HENRICI'S, though it was nearly or quite non-toxic. HENRICI had reported failure in the attempt to extract his toxin by grinding with sand.

The opportunity to prepare and study the antigenic endotoxins described by HENRICI came when numerous cultures of both *A. fumigatus* and *A. flavus* [1]) were isolated from two groups of Humboldt penguins showing widespread lesions of aspergillosis at autopsy (TILDEN et al, 1957). The investigation was initiated with a view to the possible usefulness of the toxins in immunization against the fungi and in early diagnosis of the disease, which in penguins at least had been possible only at autopsy (AINSWORTH & REWELL, 1949). Preliminary experiments with crude mycelial extracts of *A. fumigatus* confirmed HENRICI'S findings with regard to their hemolytic potency, their antigenicity, and their toxicity for rabbits, guinea pigs and mice (TILDEN et al, 1957). Our results indicated that uremia occured both in rabbits killed by the toxin and in those killed by spore injection, as shown by the accumulation of protein decomposition products in the blood before death and by the gross and microscopic findings in the kidneys. We were unable to confirm HENRICI'S experiment in which four of six rabbits immunized to the toxin resisted inoculation intravenously with spores.

The present report is concerned with the preparation of the crude toxins of *Aspergillus fumigatus* and *A. flavus* and a comparison of their biological properties, as well as preliminary experiments on concentration and purification. Experiments on further purification of fumigatus toxin will be reported elsewhere. (RAU et al, 1961).

EXPERIMENTAL METHODS

The cultures were propagated as surface mats in Fernbach flasks containing 500 ml of culture medium. Suspensions of spores (5 to 10 billion) for inoculation were obtained by first inoculating several flasks of Sabouraud's or potato dextrose agar with a heavy spore suspension harvested from a series of slants. Tween 80, 0.02 % in physiological saline, yielded homogeneous suspensions of spores suitable for counting in a hemocytometer.

Several culture media were developed, all of which were prepared in well waters which contained considerable amounts of inorganic material (Table I).

Medium 1: (TARLATZIS et al, 1957). 15 g Bacto-peptone (Difco), 6 g beef extract (Difco), 30 g dextrose, 5 g NaCl, water 1 liter. The pH was 6.8.

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[1]) We are indebted for final identification of the cultures to Miss DOROTHY FENNELL and Dr. C. W. HESSELTINE, of the Northern Regional Research Laboratory U.S.D.A., Peoria, Ill.

TABLE I.

Chemical Analysis of Water from two Wells *)
(*parts per million*)

	No. 1	No. 2
Total hardness (as $CaCO_3$)	368	436
Calcium hardness	196	240
Magnesium hardness	172	196
Alkali (phenolphthalein)	0	4
Total alkali (as $CaCO_3$)	326	364
Chloride (as NaCl)	4	10
Sulfate (as Na_2SO_4)	63	128
Total phosphate (as PO_4)	0.3	0.4
Total silica (as SO_2)	15.	14.
Total iron (as Fe)	0.3	2.1
Nitrate (as NO_3)	Trace	6.
Copper (as Cu)	0.01	0.07
Zinc (Zn)	0.2	9.0
Manganese (as Mn)	Trace	0.01
pH	7.6	7.95

*) Nalco Chemical Co., Chicago.

Medium 2: Similar to Medium 1, but with 5 g yeast extract in place of beef extract. The pH was 6.8.

Medium 3: 3 g $NaNO_3$, 75 ml corn steep liquor ¹) ,20 g dextrose, 0.015 g $ZnSO_4$, water 1 liter. The pH, which was 4.0, was adjusted with NaOH to 7.6.

Medium 4: Ingredients in 1 liter were 3 g $NaNO_3$, 10.7 g NH_4Cl, 20 g dextrose, 0.015 g $ZnSO_4$, and 5 ml each of Solutions A and B, as used in microbiological analysis by means of lactobacilli (DUNN, SHANKMAN & CAMIEN, 1945). Solution A contained in 250 ml 25 g KH_2PO_4, and 25 g K_2HPO_4. Solution B contained in 250 ml 10 g $MgSO_4.7H_2O$, 0.5 g NaCl, 0.5 g $FeSO_4.7H_2O$, and 0.5 g $MnS0_4$. The pH was adjusted to 7.6.

Medium 5: Same as Medium 4 plus 1 ml vitamin B complex ²) added to each flask, pH 7.6.

Medium 6: Same as Medium 4 plus 3 g/liter of potassium tartrate, pH 7.6.

Medium 7: Same as Medium 4 but with the NH_4Cl replaced by casein hydrolysate (enzymatic) ³), 12.5 ml to each flask, pH 6.9.

Cultures. In most of the experiments the cultures used were those recovered from rabbits infected by intravenous inoculation of spores of a culture originating from one of the Humboldt penguins.

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¹) A. E. Staley Mfg. Co., Decatur, Illinois.

²) Vi-B-Complex Solution with B 12, Haver Glover Laboratories, Kansas City, Mo.

³) "Vitamin-Free" Casein Hydrolysate, Nutritional Biochemicals Corp., Cleve land, O.

In addition, two cultures of *A. fumigatus* of mammalian origin were studied for toxin production, one from pulmonary aspergillosis in a blesbok (*Damaliscus albifrons*) and one from man [1]). A culture from a Brandt goose was also compared with the penguin culture [2]).

Extraction of toxins. The culture medium was decanted from the flasks and the mats well washed with distilled water, then drained on towels and reduced to small particles by passing twice through a Foley food mill. Finally, they were ground for approximately 30 minutes in a Sargent wet mill. They were usually stored for 1 to 4 days at—15° before grinding.
McIlvaine buffer was added before grinding, pH 7.6 for fumigatus mats and pH 5.5 for flavus mats, the volume of fluid being approximately 50 to 60 ml for each 100 g of mycelium. The moist mass of ground mycelium was stored at 4° for 5 days, a period indicated as optimal by comparison of several small batches of the same lot, then centrifuged to remove the mycelium. The extract was adjusted to pH 7.0 and immediately filtered through a Seitz pad ST 1. The less potent second and third extracts were combined, after testing, with similar extracts from other lots for purification. All extracting and diluting fluids were prepared in "water for injection", as supplied by various drug firms, to avoid the possibility of introducing bacterial endotoxins (BURROWS, 1951). Aseptic conditions were maintained up to the time of removal of the mats, and bacteriologically clean technique was used throughout.

Hemolytic assays. Sheep erythrocytes preserved in Alsever's solution and kept at 4° not longer than 2 weeks were washed with physiological saline and diluted to give a 2 % suspension. To dilutions of the toxin in a volume of 1 ml were added 1 ml of the cell suspension. The amount of fungal extract which produced complete hemolysis in 2 hrs. (30 minutes at 40° and 1½ hours at room temperature) was taken as one hemolytic unit.

Hemagglutination tests (SAB Manual, 1957, p. 269). Rabbit erythrocytes, preserved in Alsever's solution at 4° and washed with physiological saline, were prepared in 2 % suspension, and this was diluted to 0.25 %. Dilutions of the fungal extract from 1:1 to 1:1024 were prepared in a volume of 0.5 ml, and to each dilution was added 0.5 ml of the 0.25 % suspension of the rabbit cells. The amount of the fungal extract which produced complete agglutination of the cells in 2 hours at room temperature was taken as one HA unit.

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[1]) Received through the kindness of Dr. JOHN ULRICH, of the Mayo Clinic.

[2]) The blesbok culture, though somewhat atypical in the appearance of its spores, was diagnosed as *A. fumigatus*, both by Dr. LUCILLE K. GEORG, of the Communicable Disease Center, Atlanta, Ga., and by Miss DOROTHY FENNELL, now at the U.S. Quartermaster Depot, Natick, Mass.
The culture from the Brandt goose was identified at C.D.C.

Assays for toxicity. Male Swiss albino mice weighing 25 to 30 grams were injected intravenously (tail vein). The doses of fumigatus extracts were chosen on the basis of a preliminary hemolytic assay, 2 and 1 toxic units of the crude extracts corresponding to 10 and 5 hemolytic units. Pairs of mice were used for each dose, six mice for each dose in determining the LD_{50} dose. A preliminary hemagglutination test was carried out in the case of the flavus extracts; if the HA titer was 1:256 or greater, a toxic potency of 10 or more units/ml was usually found. The dose of crude extract required to kill the mice in a period of 3 to 6 days was taken as one toxic unit; occasionally a mouse died as late as 7 days, in which case 1 unit was recorded. Two toxic units of fumigatus toxin killed in 24 to 48 hours. The same survival periods have been used in recording the toxic units of flavus toxin. The toxicity of lyophilized toxins was recorded as the number of toxic units/mg.

Assay of culture fluids. One liter of culture fluid was saved from a given batch of cultures, dialyzed, concentrated by rapid evaporation by electric fan at room temperature in the dialyzing sac, and lyophilized. The dried material was dissolved in 20 % concentration in physiological saline. Further concentration was achieved, in the case of the fumigatus, by precipitating the concentrated solution with acetone in equal volume at low temperature and dissolving the precipitate in 10 ml of physiological saline.

Immunization of rabbits. Rabbits were injected intraperitoneally at 4 to 5 day intervals with fumigatus toxin, beginning with a dose of 10 toxic units and increasing very gradually to 500 units. After purified preparations became available (RAU et al, 1961), the series of intraperitoneal injections were followed by a series of intravenous injections, beginning with a dose of 5 to 10 toxic units and increasing to 2,000 to 3,000 units. The flavus toxin was injected intravenously in the crude form after a few intraperitoneal injections, 500 toxic units being the largest dose injected.

Neutralization tests. Dilutions of the immune serum from 1:1 to 1:256 were prepared, and to each dilution was added an amount of toxin corresponding to twice the LD_{50} dose as previously determined. The proportions were such that each of 6 mice received 0.5 ml of serum dilution and 0.1 ml of toxin intravenously (tail vein). Mice of 25 to 30 g were used. The mixtures were allowed to stand in the refrigerator for 18 to 24 hours. The mice were autopsied as soon after death as possible. Death and the typical renal necrosis, recognizable grossly, were recorded. The results, calculated according to REED & MUENCH's method (1938), were plotted on logarithm paper, serum doses against percentage survival.

Precipitin tests. The dilution of antigen of "optimal proportions" was first determined and this amount tested with dilutions of serum (SAB Manual, 1957, p. 209).

Inhibition of hemolysis was determined by adding varying amounts of serum to parallel series of hemolytic tests, set up as previously outlined.

Inhibition of hemagglutination was determined by the usual method (SAB Manual, 1957, p. 271).

Acetone precipitation. An equal volume of acetone was added to the crude toxin which had been adjusted to pH 6.0 with N/1 HCl. Both toxin solution and acetone were cooled to—15° before mixing. The temperature was kept low (0° to 4°) and the contact with acetone brief. The precipitate formed quickly and could be thrown down in the centrifuge in 10 minutes at 2,000 RPM. It was dissolved in physiological salt solution and tested for potency, then dialyzed against 20 volumes of distilled water in the refrigerator for 3 days with 5 changes of water, and finally lyophilized.

Precipitation with ammonium sulfate. Solutions of crude toxin were adjusted to pH 4.0 Some inert material separated, especially from the fumigatus solutions. After this had been removed by centrifugation, the supernatant was saturated with solid ammonium sulfate and allowed to stand one or two hours before centrifugation. The supernatant was seldom clear. It was withdrawn with a large volumetric pipette and passed through a fluted filter paper by gravity. The precipitate in the centrifuge bottle was suspended in water and the small amount of sedimented material on the filter paper washed into the main solution, which was then transferred to viscose tubing for dialysis against 20 volumes of distilled water at 4° for 3 days with two changes each day. The dialyzed solution was freed from any precipitate forming during dialysis and lyophilized.

Skin tests. Toxin solutions were diluted to contain the desired number of toxic units (as determined in mice) in 0.1 ml and injected intracutaneously with a 27 gauge needle. When immune serum was to be tested, it was mixed in equal parts and 0.2 ml of the mixture introduced, the control solutions being diluted with an equal part of physiological saline.

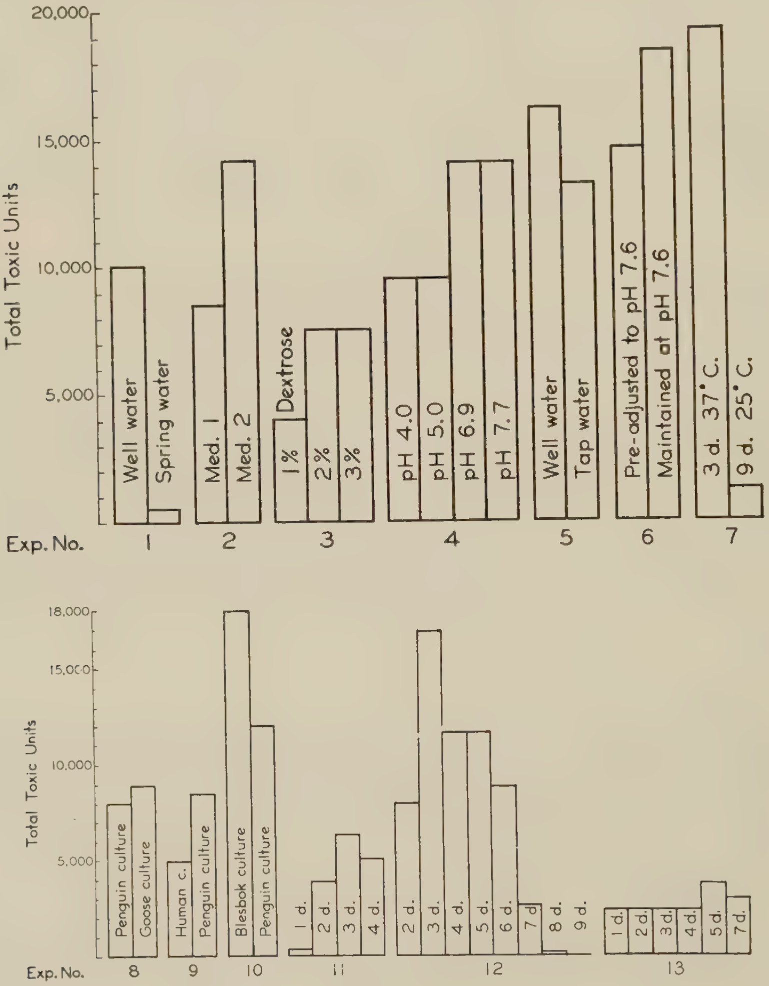
Nonprotein nitrogen was determined by the microkjeldahl technique, with nesslerization of the distilled ammonia as a means of nitrogen quantitation (HAWK, OSER & SUMMERSON, 1954, p. 830).

Inorganic phosphate was determined by the Fiske and Subbarow method (HAWK, OSER & SUMMERSON, 1954, p. 630).

RESULTS

Production of toxin of A. Fumigatus (Exper. 1-13)

Figs. 1 and 2 summarize the experiments carried out to establish the optimal conditions for production of the toxin of *A. fumigatus*. A twenty-fold increase in potency over that reported by HENRICI was obtained by supplementing the medium he used (peptone and



Figs. 1 and 2. Conditions for production of toxin of *A. fumigatus*. Each experiment represents a comparison carried out with the same number of culture flasks (6) inoculated with a measured amount of spore suspension of previously determined spore content. Total toxic units = toxic units/ml. times volume of extract.

dextrose) either with beef extract, as used by TARLATZIS, PANETSOS & DRAGONAS (1957) (Medium 1), or with yeast extract (Medium 2). Eventually it was found that a corn steep medium similar to one of those devised by MOYER (1948) for penicillin production, but with substitution of dextrose for lactose (Medium 3) and adjustment of the pH to 7.6, yielded toxin with as high as 200 toxic units/ml for the mouse, as compared with 5/ml reported by HENRICI and 100/ml for media 1 and 2. The inorganic constituents of the well waters used (Table I) were evidently important, since purified spring water [1]) was ineffective either for growth or for toxin production (Exper. 1). Tap water could be used but was not equal to well water (Exper. 5). Medium 2 was more favourable than Medium 1 (Exper. 2). Dextrose in 1 % concentration was inadequate, but there was no difference in yield between 2 % and 3 % (Exper. 3). Exper, 4 showed that adjustment of Medium 3 from pH 4.0 to a neutral or alkaline reaction made it considerably more favorable for toxin production. Maintenance at pH 7.6 increased production beyond that obtained by adjusting before inoculation (Exper. 6). Exper. 7 confirmed an earlier observation that room temperature was not favorable for toxin production. The growth attained in 9 days at 25° was adequate; the mats actually proved somewhat heavier than those taken after 3 days at 37°, but very little toxin could be extracted from them.

Moderately potent toxin was produced in cultures grown on Mediums 4, 5, and 6, which were based on Medium 3, the organic nitrogen being replaced by ammonium chloride supplemented by other salts, such as are used in the medium for microbiological assay by means of lactobacilli (DUNN, SHANKMAN & CAMIEN, 1945). Replacement of the ammonium salt with casein hydrolysate in Medium 7 yielded almost no toxin. Since the mats were well washed with distilled water before extraction, the presence of organic matter in the medium was not regarded as of great consequence, and further studies of synthetic media were not made.

Experiments 8, 9, and 10 show that cultures of *A. fumigatus* from species other than the penguin were active in producing the toxin. Dr. ULRICH's human culture, when received, had not sporulated, but after several successive passages on potato dextrose agar sufficient spores could be obtained for inoculation of the culture flasks. The other culture of mammalian origin (Exper. 10) produced toxin of even higher potency than did the penguin culture.

The optimal incubation period for formation of fumigatus toxin was 3 days, as first indicated with the use of the less favorable Medium 1 (Exper. 11) and confirmed later with Medium 3 (Exper. 12). Storage of the ground mycelium definitely increased the potency of the extract (Exper. 13), hence a period of 5 days at 4° was used routinely. No difficulty was experienced with bacterial contamination while the extract was in contact with the ground mycelium. Several antibiotic substances are produced by *Aspergillus fumigatus* (Anslow & Raistrick, 1942; Chain, Florey, Jennings & Williams, 1943; Waksman, Horning & Spencer, 1943; Waksman & Geiger, 1944) and *A. flavus* (Jones, Rake & Hamre, 1943; White & Hill, 1943; Bush & Goth, 1943; McKee, Rake & Houck, 1944). Perhaps these are present in the mycelium at this stage. Once the extract was separated by centrifugation, Seitz filtration was necessary. No loss of potency occurred if the extract was adjusted to pH 7.0 before filtration. Crude preparations of fumigatus toxin, if sterile, have been found to retain their potency for at least two years, if kept at 4°.

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[1]) HINCKLEY & SCHMIDT's "Corinnis water".

Filtrates of the culture fluid after removal of the mats were reported by Henrici to give negative tests for hemolytic and toxic action. This finding was confirmed. However, by concentrating a liter of culture fluid twenty-fold and making a 20 % solution of the lyophilized material, it became possible to demonstrate that the liter of culture fluid contained 650 hemolytic units. Further concentration by acetone precipitation and solution of the precipitate in 10 ml of physiological saline yielded a preparation with sufficient toxic potency for testing in mice; it was found that the liter of culture fluid contained 119 toxic units. The extract of the mats, which had a volume of 620 ml, contained more than 30,000 toxic units.

Production of Toxin of A. Flavus (Fig. 3, Exper. 14-18)

Moderate success has been attained in producing the toxin of *A. flavus* by culturing on Medium 1 or Medium 2. The toxic potency of the preparations obtained has not exceeded 50 toxic units/ml. Lactose did not substitute for dextrose in Medium 1 (Exper. 17). The pH of the medium was not critical so long as it was not too low (Exper. 16), nor was the temperature of cultivation critical so far as concerned the amount of toxin produced (Exper. 15). However, considerable amounts of dark pigments [1]) formed during processing of mats grown at 37°. Since these did not form when the cultures were incubated at 25°, the latter temperature was found preferable, though the period of incubation required was longer. The pH of the buffer used in extraction was evidently not critical (Exper. 18). Media 4 and 7 were unfavorable (Exper. 14).

The optimal period of incubation for toxin production cannot be defined exactly for *A. flavus*; it varied between 3 and 5 days after the surface of the medium was covered by a thin mat. An inoculation of at least 5 billion spores/flask is required if the surface of the medium is to be covered within 24 hours.

Although it was not possible to demonstrate flavus toxin in the

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[1]) These had certain characteristics of melanin pigments, being insoluble in water or organic solvents but soluble in ferric chloride.

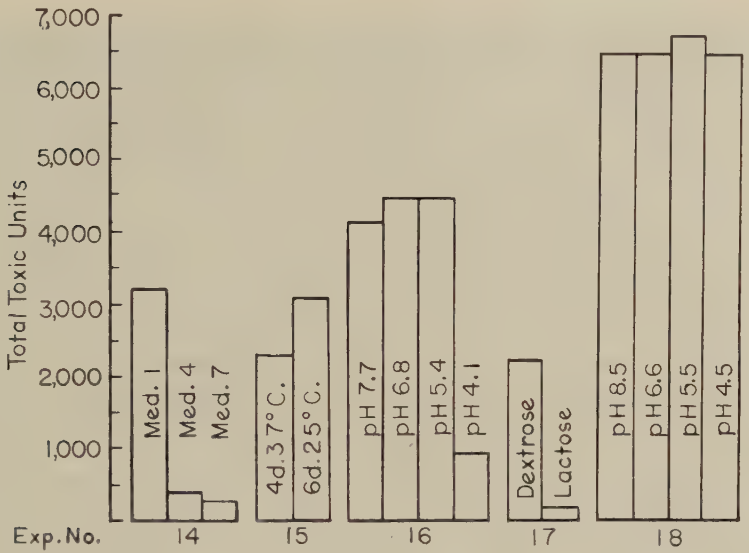


Fig. 3. Conditions for production of toxin of A. *flavus*, the comparisons made in the same way as for Figs. 1 and 2.

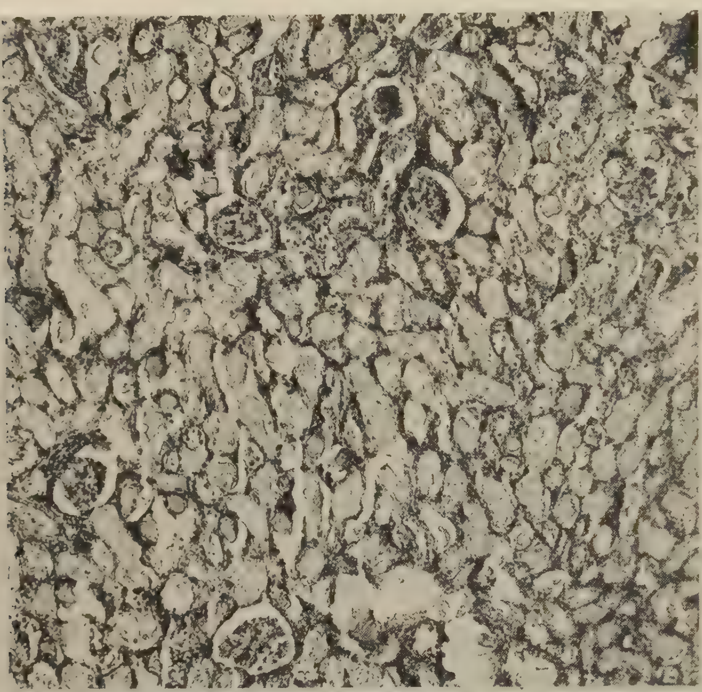
culture fluid without concentration, a content of approximately 100 toxic units/liter was demonstrated by lyophilizing a liter of fluid and dissolving the dry solids in 50 ml. The extract of the mats (700 ml) contained 4630 toxic units, or 6600 per liter, 66 times as much as the culture filtrate.

Properties of the Toxins

Toxin of Aspergillus fumigatus

The pathological effects have been studied in mice, rabbits, guinea pigs, rats, dogs, and baboons. Intraperitoneal injection is uncertain and irregular in its results, but intravenous introduction has proved dependable, and in mice it has been used for routine evaluation of toxic potency. Renal cortical necrosis is readily recognized at autopsy and may be the only gross abnormality observed. Streaks of extravasated blood where hemorrhage has occurred may be seen in the cortex, which is very pale, in marked contrast to the dark red medulla.

Histological studies of the kidney tissues of the mice shows marked destruction of the kidney cortex (Plate I, Fig. 4). The cells of the cortical tubules are flat, do not stain well, and the tubules are dilated and filled with hyaline material. The glomeruli are shrunken, and there is a wide space between the tuft and the outer border. In rabbits and dogs the changes are similar, and numerous he-



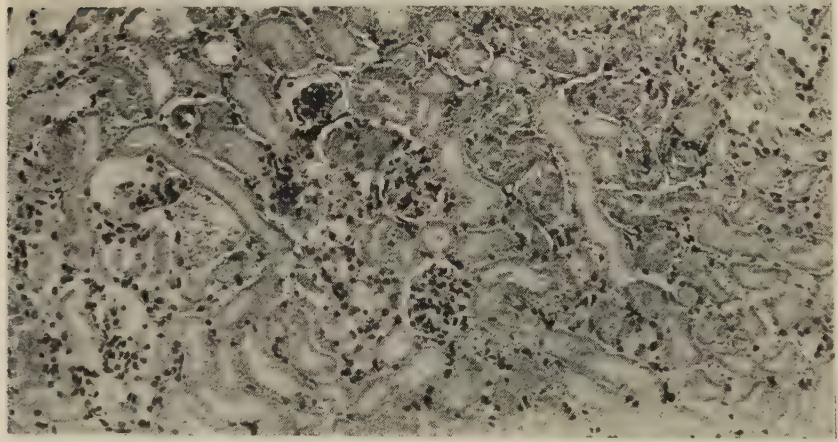


Fig. 4. (Plate I). Kidney of mouse injected intravenously with 2 toxic units of crude fumigatus toxin. Killed when moribund 48 hours after injection. × 100.

Fig. 5. (Plate I). Kidney of mouse dying 22 hours after intravenous injection of 2 toxic units of crude flavus toxin (dialyzed). × 90.

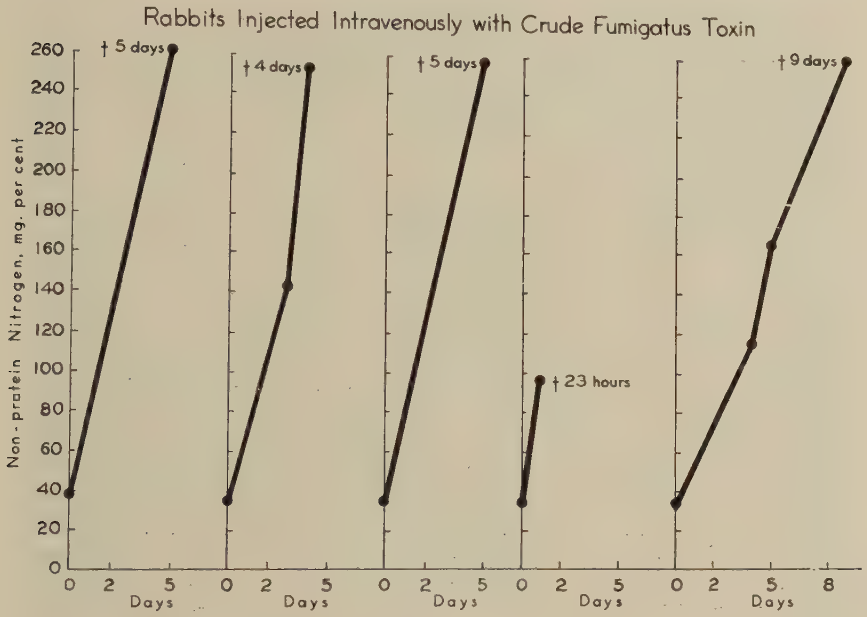


Fig. 6. Increase in nonprotein nitrogen in the blood of rabbits after intravenous injection of 5 to 10 toxic units/kg. of crude fumigatus toxin.

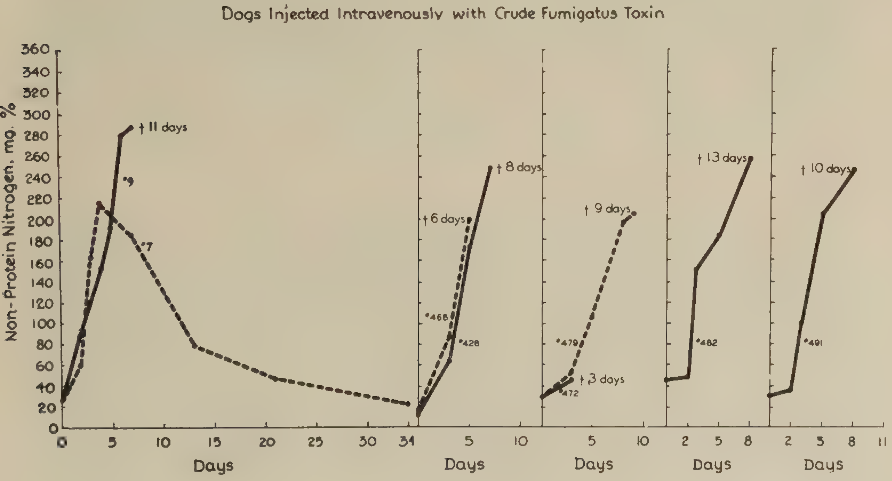


Fig. 7. Increase in nonprotein nitrogen in the blood of dogs after intravenous injection of 1 to 12 toxic units/kg of crude fumigatus toxin.

morrhages into tubules and glomeruli may be found. [1]) In rabbits and guinea pigs the liver sometimes shows fatty changes. Hemorrhages in lungs and spleen may also be found, especially in dogs, and sometimes small areas of consolidation in the lung.

Azotemia has been demonstrated in rabbits and dogs (Figs. 6 and 7). Inorganic phosphate, when determined, was also found to be greatly increased.

The fumigatus toxin is hemolytic for sheep erythrocytes. and to a lesser extent for the erythrocytes of other animals and of man (Table II), and preliminary tests with sheep cells have proved useful for indicating the toxic dose for mice. There is some evidence, however, that the hemolytic and nephrotoxic properties are not due to the same agent. In the crude extracts the ratio of hemolytic to toxic activity is regularly 5:1, i.e., 5 hemolytic units are equivalent to 1 toxic unit. When such preparations are heated for 5 minutes at 70°, the ratio is 5:4, and after 60 minutes at 60°, the number of toxic units in 1 ml is twice the number of hemolytic units (Table III). On the other hand, in preparations which have been partially purified by repeated precipitation with acetone, the ratio of hemolytic to toxic units is often 10:1, the hemolytic property being apparently more stable to treatment with acetone than the toxic (RAU et al). Further purification of the toxin may resolve this problem.

The fumigatus toxin has marked dermonecrotic activity, as seen in the rabbit skin. Injection of 0.1 ml containing 2 toxic units for the mouse leads in a few hours to swelling over an area 2 to 3 cm in diameter. At the center of this is a necrotic area 0.5 to 1.0 cm in diameter surrounded by a zone of erythema. When the inoculum contains 5 toxic units there is much hemorrhage just inside the border of the necrotic area. The animal's temperature may rise 2 or 3 degrees F. during the first 24 hours but soon returns to normal. The lesions heal slowly. Within a week the necrotic area shows a scab, surrounded by slight redness and induration, but little change occurs in the next two or three weeks.

Toxin of Aspergillus flavus

The flavus toxin is also a nephrotoxin, and histological studies of kidney tissues of mice killed by it show a marked similarity in pathological changes to those induced by the fumigatus toxin (Plate I, Fig. 5). In other respects, however, there are striking differences. No hemolytic activity has been demonstrated for the flavus toxin. It possesses a potent hemagglutinin for rabbit erythrocytes, but there is no constant parallelism between hemagglutinative and toxic potency such as was found between hemolytic and toxic potency of crude fumigatus preparations. Hemagglutinative activity

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[1]) Similar, though less severe kidney lesions have been observed in birds dying of spontaneous aspergillosis.

TABLE II.

Effect of Fumigatus Toxin on the Erythrocytes of Various Species

Species		Units of Fumigatus Toxin *)
Sheep	*Ovis aries*	1.0
Aoudad	*Ammotragus lervia*	1.0
Wildebeest	*Connochaetes gnou*	1.5
Hen	*Gallus gallus*	1.5
Peccary	*Tayassu angulatus*	5.0
Man	*Homo sapiens*	10.0
Cat	*Felis domesticus*	25.0
Northern sea lion	*Eumetopias jubata*	25.0
Sitatunga	*Tragelaphus spekei*	25.0
Rabbit	*Oryctolagus cuniculus*	25.0
,,		65.0
,,		70.0
,,		150.0
,,		150.0
,,		200.0
Dingo	*Canis familiaris dingo*	150.0
Dog	*Canis familiaris*	> 500.0
Coyote	*Canis latrans*	100.0
Harbor seal 1	*Phoca vitulina*	100.0
Harbor seal 2	,, ,,	100.0
Monkey	*Cercopithecus l'hoesti*	> 700.0
Pony	*Equus caballus*	> 500.0
Zebra	*Equus grevyi*	> 1000.0
Baboon	*Papio papio*	1000.0
Wolf	*Canis lupus*	> 1000.0

*) No. of units/ml of the toxin required to hemolyze completely 1 ml of 2 % suspension of sheep erythrocytes in 30 min. at 40° followed by 1½ hrs. at room temperature.

TABLE III.

Effect of Heating on Toxin of A. fumigatus

	Before heating	70° 5 min.	70° 10 min.	60° 30 min.	60° 60 min.
Hemolytic units/ml *)	500	25	0	5	5
Toxic units/ml **)	100	20	0	10	10
Ratio HU/TU	5 : 1	5 : 4		1 : 50	1 : 2

*) 1 unit hemolyzes 1 ml 2 % sheep r.b.c. (reading taken after 30 min. at 40° followed by 1.5 hrs. at room temp.).

**) 1 unit kills mice in 48 to 72 hrs.

was also demonstrated for the erythrocytes of the zebra (*Equus grevyi*), but not for those of monkey (*Cercopithecus l'hoesti*), wildebeest (*Connochaetes gnou*), seal (*Phoca vitulina*), sitatunga (*Tragelaphus spekei*), chicken (*Gallus gallus*), sheep (*Ovis aries*), wolf (*Canis lupus*), or pony (*Equus caballus*).

Even relatively large doses of flavus toxin (8 T.U. for the mouse) fail to induce the severe dermonecrotic reaction which cocurs when 2 T.U. of fumigatus toxin are injected intradermally.

Immunological Studies of the Toxins

Henrici reported that rabbits actively immunized against fumigatus toxin were able to withstand several times the lethal dose of the homologous toxin but not that of *A. flavus*. Our experiments show considerable immunological specificity of the two toxins in precipitin tests (Table IV), and especially in neutralization tests (Fig. 8). The severe dermonecrotic reaction induced in the rabbit skin by 2 mouse units of fumigatus toxin is neutralized by antifumigatus serum, but not by antiflavus serum.

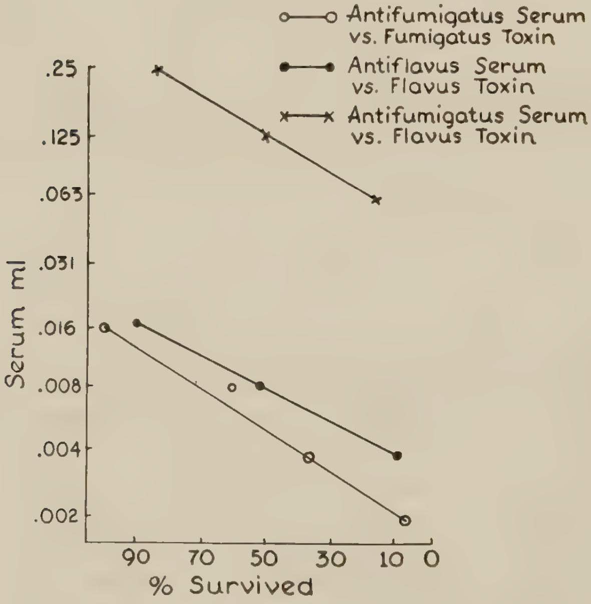

Fig. 8. Neutralization tests. Comparison of potency of immune sera against homologous and heterologous toxin.

TABLE IV.

Precipitin Tests

Serum Diln.	Antiflavus Serums vs. Flavus Toxin			Antiflavus Serums vs. Fumigatus Toxin		
	# 92	# 96	# 97	# 92	# 96	# 97
1 : 1	++++	++++	++++	++	>+	++
1 : 2	++++	++	+++	+	+	+
1 : 4	++	+	++	—	—	—
1 : 8	+	+	+		—	—
1 : 16	+	—	—			
1 : 32	—					

TABLE V.

Inhibition of Hemolytic Activity of Fumigatus Toxin *)

Serum	Fumigatus Toxin			
	100 H.U.	40 H.U.	10 H.U.	1 H.U.
Antifumigatus				
# 84, 0.5 ml	No H			
# 84, 0.25 ml	No H			
# 84, 0.1 ml	CH		No H	
# 91, 0.5 ml	No H			
# 91, 0.25 ml	CH	No H		
# 89, 0.25 ml	No H			
Antiflavus				
# 92, 0.5 ml	CH	CH	CH	CH
# 97, 0.5 ml	CH	CH	CH	CH
Pre-Immunization				
# 91, 0.5 ml	CH	CH	CH	CH
# 84, 0.5 ml	CH	CH	CH	CH
# 92, 0.5 ml	CH	CH	CH	CH
# 97, 0.5 ml	CH	CH	CH	CH

*) *vs. Sheep Erythrocytes*, 1 ml. of a 2 % suspension
No H — Complete inhibition of hemolysis
CH — Complete hemolysis, no inhibition

TABLE VI.

Specific Inhibition by Homologous Immune Serum of Hemagglutination due to Flavus Toxin

Flavus Toxin *) vs. Rabbit Erythrocytes

	No Serum	Antiflavus Serum # 92	Antifumigatus Serum # 84	Pre-Immunization Serum # 92
HA titer **)	128	0	128	128

*) Diluted to contain 16 toxic units.
**) Reciprocal of highest dilution of toxin causing agglutination.

Hemolysis of sheep erythrocytes by the fumigatus toxin is also prevented by antifumigatus serum but not by the serum of the same rabbits taken before immunization nor by antiflavus serum (Table V). The hemagglutination of rabbit erythrocytes by the flavus toxin, similarly, is inhibited by antiflavus serum but not by preimmunization serum or by antifumigatus serum (Table VI).

Chemical Studies of the Toxins

It was possible to concentrate small amounts of crude fumigatus toxin by careful distillation *in vacuo* at 30—35° without loss of potency. Potency was not affected by Seitz filtration at pH 7, by dialysis, by freezing and thawing, or by lyophilizing. Crude preparations retained their potency for at least 2 years at 4°. Trial of several organic solvents, methyl, ethyl, and isopropyl alcohols and acetone, showed that the last two were most efficient in precipitating both the hemolytic and toxic substances. As shown in Table VII, dialysis alone resulted in tenfold concentration, much more than with acetone precipitation alone, but the greatest activity was obtained if the crude extracts were first precipitated with one volume of

Table VII.

Potency of Preparations of Fumigatus Toxin
Effect of dialysis and precipitation with acetone

Prep. No.	Vol.	H.U.	T.U.	Vol. acetone	Wt. after lyophilizing	H.U.	T.U.	
	ml	/ml	/ml	ml	g	/mg	/mg	
1A	80	500	100	none	5.2759	7.6	. . .	Not dialyzed
1B	200	500	100	200	0.5280	190.0	19	Dialyzed vs. distilled H_2O
1C	Supernatant from 1B			400	0.1940	1.3		Dialyzed vs. distilled H_2O
2A	90	500	100	none	7.1382	6.3	. . .	Not dialyzed
2B	84	500	100	none	0.5409	77.6	. . .	Dialyzed vs. distilled H_2O
2C	200	500	100	200	0.5005	200.0	20	Dialyzed vs. distilled H_2O
3A	350	300	60	350	2.9144	32.5	3.25	Not dialyzed
3B	350	300	60	350	1.0382	90.9	18.2	Dialyzed vs. M100 NaCl
3C	350	300	60	350	0.9420	117.0	23.4	Dialyzed vs. Distilled H_2O

acetone and then dialyzed. Precipitation with 2 volumes of acetone did not increase hemolytic or toxic potency (1C). Several liters of crude toxin were processed by the method used for 3C (Table VII) and combined for chemical investigations (RAU et al). Later it was found that precipitation of the toxin by saturation with ammonium sulfate followed by dialysis and lyophilization yielded preparations of higher toxic potency for mice. (34 to 40 T.U./mg.).

The potency of flavus toxin was not affected by passage through a Seitz filter (ST 1) at pH 7.0, nor by dialysis, freezing and thawing, or lyophilization. On storage at 4°, however, it was much less stable than fumigatus toxin, the losses in toxic potency in a period of 4 to 6 weeks being as much as 50 per cent in some instances. No toxicity could be demonstrated in crude preparations heated at 60° for 15 minutes. None of the solvents tried produced an active precipitate from crude extracts, but precipitation by ammonium sulfate and dialysis of the aqueous solution of the precipitate, followed by lyophilization, has yielded dry preparations which have remained stable for at least two months.

DISCUSSION

The widespread occurrence of endotoxins in Gram-negative bacteria has led to extensive studies of their nature and properties (BURROWS, 1951), but the association of endotoxins with fungi is less well established. The discovery of endotoxins in *Aspergillus fumigatus* and *A. flavus* by HENRICI (1939) has been widely quoted as possibly furnishing a basis for explaining the mechanism by which *A. fumigatus* produces severe disease in birds, but until the present study was undertaken no attempt had been made to confirm his work.

It was not found possible to prepare toxins of sufficiently high potency by the method described by HENRICI. However, the extracts we have prepared have most of the properties he ascribed to the aspergillus toxins. They are obviously endotoxins, since we found the culture fluids relatively devoid of toxicity as compared to the fluid expressed from the mycelia. Our preparations, like HENRICI'S, were heat labile, differing in this respect from the typical bacterial endotoxin. The hemolytic activity observed for the fumigatus toxin and its absence from the flavus toxin are other properties common to HENRICI'S preparations and ours.

Neutralization tests have shown that the fumigatus and flavus toxins are distinguishable immunologically. Intradermal tests, conducted with preparations of similar toxic potency for mice, permitted us to differentiate the severe necrotic lesion induced by fumigatus toxin from the slight inflammatory reaction given by flavus toxin. Finally, a comparison of chemical properties suggests possible differences.

The chemical nature of the fumigatus toxin will be discussed elsewhere (RAU et al). The rapid rise in nonprotein nitrogen and phosphate in the blood following intravenous injection of fumigatus toxin suggests renal failure as the probable cause of death. Transient azotemia may be produced by sublethal doses of fumigatus toxin (Fig. 7, Dog 7). Extensive tubular necrosis and hemorrhage into the cortex of the kidney were the usual microscopic findings when lethal doses of the toxin were injected, and these proved to be the regular result of severe toxic disease in mice and rabbits, whether induced by fumigatus toxin or by flavus toxin. Histological studies of other tissues in mice, dogs, and rabbits, showed varying degrees of pathologic effects in liver, spleen, heart, and lungs, but none of these occurred with the consistency of the nephrotoxic action.

There seems little doubt that two distinct toxic substances have been produced, under somewhat different cultural conditions, one by *Aspergillus fumigatus* and one by *Aspergillus flavus*.

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SUMMARY

Endotoxins have been reported to occur in *Aspergillus fumigatus* and *A. flavus*, but the data regarding them are incomplete. Optimal cultural conditions and methods of extraction are described here for the production of the toxins of both of these fungi. They are obtained from the well-washed mycelia; only minute amounts could be demonstrated in the concentrated culture fluids. Both are powerful nephrotoxins; a characteristic necrosis of the kidney cortex is evident grossly in mice, and histological study shows no difference between the effects on the kidney of the two toxins. However, the fumigatus extracts have strong hemolytic properties, especially for sheep erythrocytes, and marked dermonecrotic activity in rabbit skin. No hemolytic action has been demonstrated for the flavus extracts, which possess a powerful hemagglutinin for rabbit erythrocytes and only mild dermonecrotic properties. Immune sera have been prepared in rabbits for both toxins, and in each case these prevent all the observed effects of the toxin. Precipitin titers are closely correlated with neutralizing titers. Chemical differences are suggested by the different procedures applicable to concentration and purification of the toxins. The toxin from *A. fumigatus* is precipitated by acetone and can be concentrated and purified to a considerable degree by acetone precipitation and dialysis. The flavus toxin has not been precipitated by any of the solvents tried but can be freed to some extent of inert substances by acid treatment and saturation with ammonium sulfate.

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PARTIAL PURIFICATION AND CHARACTERIZATION OF THE ENDOTOXIN FROM ASPERGILLUS FUMIGATUS [1]

by

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(with 3 figs.)

(12.XII.1960)

INTRODUCTION

As a sequence to the work described in a paper by TILDEN et al. (1961) on the properties of the endotoxin from *Aspergillus fumigatus*, an investigation dealing with the further purification and characterization of the toxin has been undertaken. Since a review of the early work on this toxin has been covered (TILDEN et al., 1961), no attempt will be made to repeat a survey of the literature. This toxin manifests both toxic and hemolytic properties (TILDEN et al., 1961). Attempts to further purify the crude toxin were by the methods of fractional precipitation with acetone, chromatography on DEAE cellulose, and column electrophoresis. Characterization of the material was by electrophoresis.

MATERIALS AND METHODS

The starting material for the studies described in this investigation was the toxic extracts of *A. fumigatus* mycelia described by TILDEN et al. (1961). These extracts represent a crude preparation of the toxin, though highly potent. Since acetone was found to precipitate the toxic and hemolytic agents from aqueous extracts (TILDEN et al. 1961), the use of acetone was further investigated. The pH of the crude toxin extract was adjusted from 7.0 to 6.1

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by adding 1.0 M HCl slowly with stirring at room temperature. The volume of the adjusted extract was measured, and the extract was poured into a beaker sufficiently large to hold an addition of one volume of acetone. The extract was cooled to 0° C. An equal volume of cold acetone (-10 °C) was added slowly through a capillary with thorough mechanical stirring. The addition of acetone to the extract took place in a cold room (4° or 5° C.). The precipitate formed was centrifuged in a refrigerated centrifuge at 2,000 r.p.m. The supernatant was discarded. The residue was suspended in a minimum of distilled water, placed in a Visking casing, and put to dialyze against a large volume of distilled water in the cold room for 24 to 36 hours. The dialysate was replaced four or five times with fresh distilled water. After dialysis, the toxin suspension was centrifuged. The insoluble residue was discarded. The supernatant was lyophilized, weighed, assayed, and stored in the refrigerator.

Dry preparations of the toxin having similar hemolytic potencies were combined and reprecipitated with acetone. To suspensions containing 1 g of toxin per 100 ml McIlvaine's buffer, pH 6.1, one volume of acetone was added as described above. The precipitates were suspended in a minimum of distilled water, dialyzed, and lyophilized as described above.

Moving boundary electrophoresis was used to follow the purification process. The Spinco Model H Electrophoresis and Diffusion Apparatus was used. Approximately 75 mg dried toxin were suspended in 15 ml phosphate buffer, pH 7.7, ionic strength 0.10. The solution was dialyzed overnight against 1500 ml of the same buffer. The electrophoretic patterns were interpreted according to the method described by KOENIG & HOGNESS (1946).

The use of DEAE-cellulose chromatography as described by SOBER et al. (1956) for the purification of the toxin was investigated. BROWN and Company's "Selectacel"-DEAE standard capacity cellulose was used. A ratio of 30.8 mg toxin per gram of DEAE-cellulose was used. The Cellulose was washed three times with 0.005 M NaCl. Three grams DEAE-cellulose were suspended in approximately 100-150 ml 0.005 M NaCl. This slurry was magnetically stirred at a slow speed in a 200-ml Erlenmeyer flask having a spigot near the bottom. A glass column, 1 cm in diameter, fitted with a perforated plate at the bottom, was filled with the slurry of DEAE by means of a tube connected to the spigot of the Erlenmeyer. Each addition of slurry to the column was stirred with a long wire to release trapped air and then allowed to settle by gravity. The packed column was washed with 200 ml 0.005 M NaCl to provide temperature and pH equilibrium. If the cellulose from a column was to be re-used, it was washed three or four times with 0.5 M NaCl, then three or four times with water, and then several times with 0.005 M NaCl. Volumes of the 200 ml were used for each washing.

The sample, suspended in a minimum volume of 0.005 M NaCl was added to the column. An additional volume of 30 ml 0.005 M NaCl was washed through the column. Elution was by the gradient method and 5 ml portions were collected on the fraction collector. Gradients were produced by introducing from a Mariotte-tube controlled separatory funnel a solution having a concentration equal to the gradient limit (0.152 M NaCl) into a constant volume mixing chamber (ALM et al., 1953; CHERKIN et al., 1953; DONALDSON et al., 1952). Flow was established from the mixing chamber, which contained initially 100 ml 0.005 M NaCl, into the column. The mixing chamber was magnetically stirred. With the exception of the uppermost separatory funnel, the system was closed to the atmosphere in order to insure equal flow rates throughout.

Column electrophoresis as described by SVENSSON & VALMET (1959) was also used to purify the toxin. The density-gradient method using sucrose with a limiting concentration of 58 % was used. The LKB Column Electrophoresis Apparatus with phosphate buffer of pH 7.7 and ionic strength of 0.01 was used. In some experiments, buffer having an ionic strength of 0.025 M was used around the anode, and additional Na_2HP0_4 was placed in the cathode chamber. The additional electrolyte prevented depletion of ions and the development of pH gradients during the course of the electrolysis. The anode and lower column were filled with buffer containing 50 % sucrose. The cathode contained no sucrose. The gradient was produced by flowing buffer containing 58 % sucrose from a reservoir through the first mixing chamber into a much smaller second mixing chamber into the bottom of the column. The first mixing chamber contained 135 ml buffer having an initial concentration of 3.625 % sucrose. The small second mixing chamber contained no more than 9 ml, usually less. The sample dissolved in buffer with no sucrose was introduced into the second mixing chamber. Both mixing chambers were magnetically stirred. The gradient was gradually flowed into the column through a capillary tube at such rate that no turbulence developed. The resulting sharp sample boundary was slowly flowed in until it reached the top of the column The flow was then discontinued, menisci adjusted, electrodes opened to the atmosphere, and electrolysis begun. The electrolysis was continued at 15 m.a. for 16 to 22 hours. A potential of 500 or 600 volts developed. The contents of the column were slowly displaced and collected in 3 ml portions on a constant volume fraction collector. The tubes were monitored by measuring optical density at 280 mμ against tubes containing only buffer. The optical density versus tube number plots indicated the location of the electrophoretic boundaries. Tubes forming various portions of the boundaries were pooled, dialyzed free of sucrose against cold water, lyophilized, and assayed for hemolytic and toxic potencies.

Biological activity consists of hemolytic and toxic manifestations. To assay for hemolytic activity, a series of dilutions of the toxin was made using 0.85 % NaCl as diluent. 1 ml of a 2 % by volume suspension of freshly washed, oxalated sheep erythrocytes was added to each of a series of test tubes containing 1 ml of the various toxin dilutions. The tubes were placed in a 40° C water bath for 30 minutes and were shaken intermittently. After removal from the water bath, the tubes were allowed to stand 2 hours at room temperature before reading. The tube having the highest dilution of toxin in which complete hemolysis occurred (no cell deposit) was designated as one hemolytic unit (HU).

To assay for toxic potency, a quantity of 0.1 or 0.2 ml of a dilution of a toxin preparation in sterile non-pyrogenic isotonic saline was injected into the tail vein of a 25-30 g male mouse. One toxic unit (TU) was defined as the smallest amount of toxin needed to kill a 25-30 g male mouse within 5 days.

Results

It was first established that freeze-drying has no deleterious effects upon the toxin. Potent toxin solutions, both crude and acetone precipitates, were freeze-dried. Assays revealed that both hemolytic and toxic potencies were recovered completely.

Acetone precipitations were carried out on small batches of crude toxin. Essentially all the toxic and hemolytic activities were found in the water soluble portion of the precipitate formed by adding one volume acetone. Thorough cooling of both the toxin solution and the acetone was necessary if good recovery of biological activity was achieved. Small acetone-precipitated batches ranged in hemolytic potency from 14.6 to 200 HU/mg. Preparations having similar potencies were pooled and re-precipitated, resulting in improved potencies.

A total of 14.7 g of combined lyophilized toxin preparations were dissolved and acetone precipitated. All constituents had been previously precipitated with acetone at least once, some as many as three times before incorporation into a large uniform sample, designated "Pool J". Approximately nine liters of crude toxin were represented in this preparation. The final product weighed 10.74 g and contained 1.4 million hemolytic units (130 HU/mg). The mouse assay revealed that 0.093 to 0.10 mg was the lethal dose for a 25-30 g mouse. This preparation was used in all subsequent experiments, because it was uniform, and subsequent assays proved it to be stable in the dry state.

Several qualitative tests were made on Pool J in an attempt to determine its chemical nature. Both biuret and Molisch tests were positive. Bial's and Tauber's (benzidine) tests were negative. The test for phosphate and the test for starch were also negative.

In order to determine the pH range for stability of the toxin, solutions of the toxin were made in buffers covering a wide range of pH and allowed to stand 48 hours at 4 or 5° C. Complete stability of the hemolytic activity was observed between pH 3.6 and

8.4. Partial or complete loss occurred outside this range.

Moving-boundary electrophoretic analysis of Pool J revealed six or possibly seven migrating components with one stationary component at pH 7.7, ionic strength 0.10. Mobilities (u) ranged from 1.16 to 7.22 $\times$ 10^{-5} cm^2 sec^{-1} $volt^{-1}$. Sedimentation patterns for the sample revealed four or five components. The Spinco Model E Analytical ultracentrifuge at 59,780 r.p.m. was used for the sedimentation analysis. Due to superior resolution by electrophoresis, the electrophoretic technique was used almost exclusively to follow purification.

Attempts to repeat HENRICI'S (1939) observation that toxic potency remained in the supernatant after precipitation of the "proteins" with trichloracetic acid were unsuccessful. Various concentrations of trichloracetic acid were used. Neither hemolytic nor toxic activity was demonstrated in the precipitate or in the supernatant neutralized with dilute NaOH.

In order to test the effect of pH on precipitation with acetone, solutions of Pool J (1 g/100 ml) were made in acetate and phosphate buffers of pH values 4.05, 5.00, 6.05, 6.90, and 7.75 and ionic strength 0.10. Each solution was precipitated with one volume of acetone. Both the precipitates and supernatants were dialyzed, lyophilized, and assayed for hemolytic activity. Full recovery was observed in precipitates at pH 4.05, 5.00, 6.05, and 6.90. At pH 7.75, 50 % of the hemolytic activity was recovered in the precipitate and about 10 % in the supernatant. Precipitation at pH 5.00 yielded the best product (208 HU/mg).

Small pilot fractionations with acetone were performed using 20 ml portions of solutions containing 1 g Pool J/100 ml acetate buffer at pH 4.0, 5.0, and 6.0 and ionic strength 0.10. Chilled acetone was added to the sample from a burette until sufficient precipitation occurred to obscure the stirring bar. The precipitate was centrifuged and suspended in distilled water. To the supernatant more acetone was added until the precipitate formed again obscured the stirring bar. Fractionation was carried out in this manner until a total of one volume acetone had been added. The resulting fractions and the assay results are tabulated in Table I. Larger samples of Pool J were fractionally precipitated with acetone at pH 4.0 and 5.0. Toxicity potencies were obtained for these fractions. The number of hemolytic units in the dose necessary to kill a 25—30 gram male mouse (HU/TU) was calculated. The results in Table II indicate that although the hemolytic and toxic activities are roughly parallel in all fractions, the ratios between the two are not constant.

Electrophoretic analyses were made on the fractions precipitated by acetone at pH 4.0 and 5.0. Mobilities and relative compositions of a series of fractions precipitated at pH 5.0 are given in Table II. To component 5 was assigned the hemolytic activity, because there was a definite correlation between the area of this component and

TABLE I.

Small-scale fractionations at pH *4.0, 5.0, and 6.0. Acetate buffers, ionic strength 0.10 were used. 0.2 g Pool J in 20 ml buffer was the sample size in each case.*

Fraction	Total volume Acetone in ml	Dry wt. of Fraction	HU/mg	Total HU in Fraction	Recover in %
			pH 4.0		
1	0.0 *)	0.0090	28.0	252	
2	10.5	0.0198	32.0	634	
3	12.0	0.0235	35.0	823	
4	14.0	0.0323	111.0	3,580	
5	17.0	0.0295	42.0	1,247	
6	22.0	0.0190	4.4	84	
				6,620	25.4
			pH 5.0		
1	8.0	0.0470	177.0	8,325	
2	11.0	0.0423	197.0	8,325	
3	15.0	0.0250	33.0	833	
4	22.0	0.0115	2.7	31	
				17,514	67.5
			pH 6.0		
1	7.5	0.0308	203.0	6,250	
2	9.5	0.0365	98.0	3,580	
3	13.0	0.0252	49.5	1,250	
4	23.0	0.0225	2.8	63	
				11,143	42.8

*) A precipitate formed spontaneously upon adjustment to pH 4.0.

TABLE II.

Electrophoretic mobilities, relative composition, and potency of fractions obtained by acetone fractionatio of 2 g Pool J pH 5.0, phosphate buffer, ionic strength, 0.10, pH *7.7, current of 16 m.a. for 214.3 mi*

	Fraction 1		Fraction 2		Fraction 3		Fraction 4		Fraction 5	
	$u \times 10^5$	%	$u \times 10^5$	%	$u \times 10^5$	%	$u \times 10^5$	%	$u \times 10^5$	%
1	7.70	8	7.35	4	7.21	3	8.47	2	6.75	4
2	6.02	18	5.88	20	5.60	15	5.60	16	5.75	11
2′									4.97	15
3	4.27	32	4.20	28	4.20	35	4.34	34	4.40	19
4					3.43	17	3.50	27	3.55	29
5	2.87	42	2.80	44	2.73	21	2.66	10		
6			1.40	4	1.61	9	1.54	11	1.78	22
HU/mg	200		266		150		27		7	
TU/mg	<20		23		15		5		2	
HU/TU	13		12		10		5		3	

the hemolytic activity of the fraction. Fraction 2 in Figure 1 has the greatest hemolytic activity (226 HU/mg) and also has the greatest enrichment of component 5. It was not possible to assign toxic activity to any electrophoretic component.

The absorption spectrum of Pool J in the ultraviolet indicated that maximal absorption occurred at 280 mμ.

Preliminary studies with DEAE cellulose indicated that the hemolytic activity was adsorbed on the cellulose when an ionic strength of 0.005 M NaCl was used. The hemolytic activity was eluted from the cellulose when the ionic strength was raised to 0.15 M NaCl. A sample of 150 mg toxin was dissolved in 0.005 M NaCl and introduced at the top of the column packed with 3 g DEAE cellulose. Elution by the density gradient method was carried out. The fractions were monitored at 280 mμ on the Beckman DU spectrophotometer. Optical density was plotted against effluent tube number. A typical curve is shown in Figure 2. One rather sharp peak was evident at a calculated molarity of 0.08 M NaCl. The fractions were pooled and designated as follows: tubes 1—8, C—1; tubes 9—40, C—2; tubes 41—100, C—3; tubes 101—113, C—4. All pools were dialyzed, lyophilized and assayed for hemolytic activity. Only C—2, which was a pool of the tubes falling within the peak, possessed hemolytic activity. A second sample of 500 mg toxin containing 100 HU/mg was chromatographed in a similar

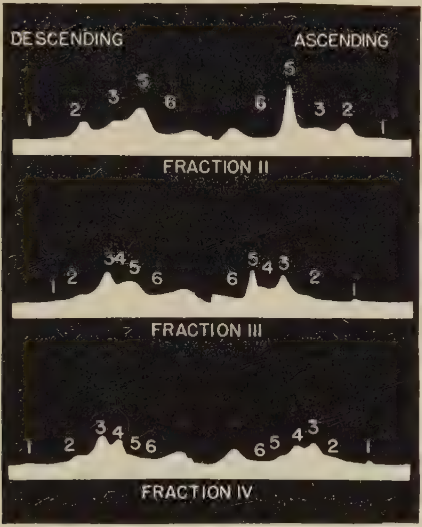

Figure 1. Electrophoretic patterns of some fractions resulting from fractional precipitation of 2 g Pool J at pH 5.0 with acetone.

manner on DEAE cellulose. Elution by the same gradient method produced a curve similar in contour to that in Figure 2. The portion of the curve corresponding to C—2 possessed hemolytic activity of 150/HUmg and toxic activity of 10 TU/mg. Less hemolytic activity was found in the other fractions corresponding to C—1, C—3, and C—4. The total hemolytic activity recovered in the fractions was over 69 % of that introduced on the column. Electrophoretic analysis of fraction C—2 revealed three components. The two major components by electrophoretic mobility were components 3 and 5 and represented 47.0 % and 46.4 % of the total area, respectively. Component 2 represented only 6.6 % of the total area.

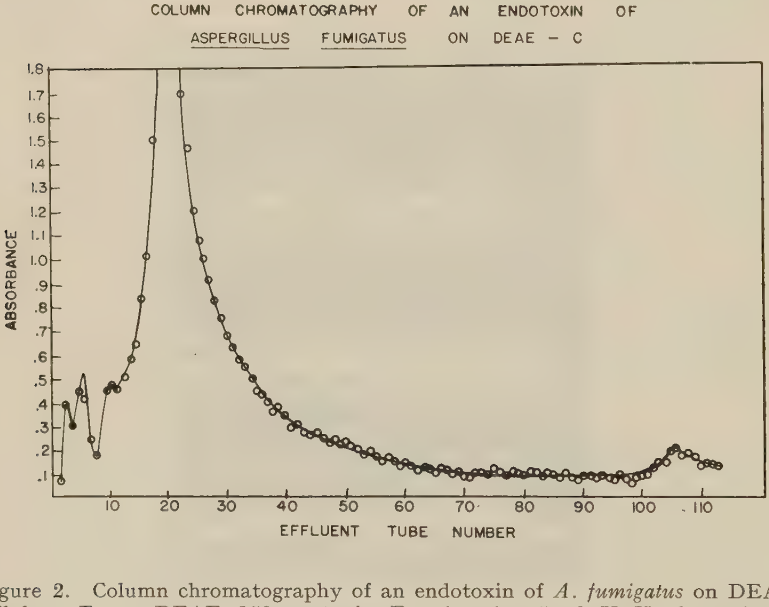

Figure 2. Column chromatography of an endotoxin of *A. fumigatus* on DEAE-cellulose. Two g DEAE, 150 mg toxin. Fraction size, 5 ml. U. V. absorption at 280 mμ.

The effectiveness of column electrophoresis was next examined. A 500 mg sample of Pool J was examined on the LKB Column Electrophoresis Apparatus using phosphate buffer, pH 7.7 and ionic strength of 0.01. The density gradient method using sucrose as described under "Methods" was used. The optical density of the effluent fractions was plotted against tube number. A typical curve is shown in Figure 3, and is similar in contour to the schlieren patterns obtained by moving boundary electrophoretic analysis of Pool J. Tubes were pooled as shown by the brackets at the top of Figure 3. Each pool was dialyzed, lyophilized and weighed. The pooled fractions, designated CE—1 through CE —7 were assayed for hemolytic and toxic activities. The results are given in Table III. Pool CE—5 corresponded to Component 5 of the moving boundary electrophoretic schlieren pattern of Pool J. The data in Table III substantiate the preliminary identification of Component 5 as the hemolytic factor and indicate that the toxic factor is closely associated with the hemolytic factor at least electrophoretically. The total hemolytic activity recovered was 29 % of that originally placed in the column. A second sample of 300 mg was examined by column electrophoresis in a similar manner. The results are also listed in Table III. The high hemolytic activity was in the same location (CE—5). The recovery of hemolytic activity in this experiment was 31 %.

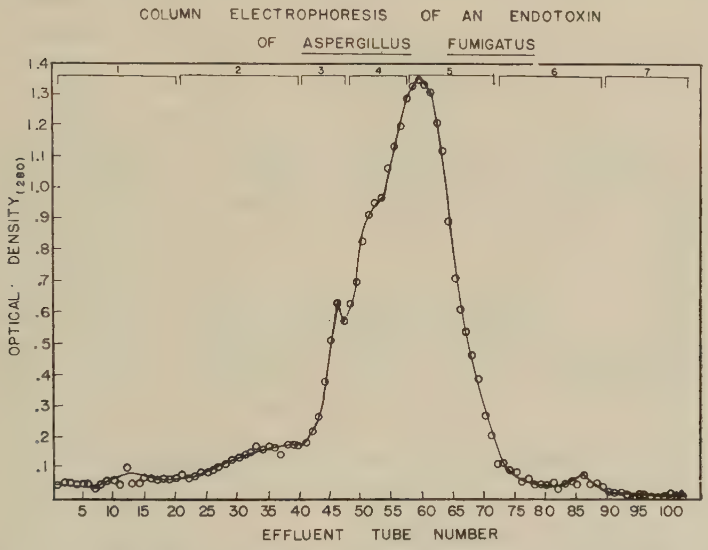

Figure 3. Column electrophoresis of 500 mg Pool J. Phosphate buffer, pH 7.7, ionic strength 0.01. Current, 0.015 amp., 500 V, 22 hours.

Discussion

Application of the acetone precipitation method to crude toxins achieved nearly a 20-fold increase in hemolytic potency on a dry weight basis. Good recovery of the hemolytic activity was usually realized, but there seemed to be some alteration of the toxic property, as evidenced by the change in the number of hemolytic units per toxic unit. Since there was at least a ten-fold enrichment of toxicity to be gained by acetone precipitation the method was used as a means of purification. Other advantages of the acetone-precipitated toxin were: (a) ready solubility of the precipitate in water, (b) greater stability without danger of bacterial growth, (c) convenience of storage, and (d) better immunity producing ability in rabbits (TILDEN et al., 1961). It is quite evident that as purification proceeds by the acetone fractionation method, recovery decreases.

The use of DEAE cellulose column with gradient elution indicated that some purification could be accomplished. The recovery was about 69 %, and can be considered rather satisfactory. There was, however, no apparent separation of the toxic and hemolytic properties. Re-runs of fractions under a variety of elution conditions might be more effective.

The column electrophoresis method certainly identified the hemolytic activity with a definite electrophoretic component. Quantities of 300 mg could be satisfactorily processed by this method; however, recovery of hemolytic activity was disappointingly low. Loss of hemolytic activity may be due to the long time for dialysis necessary to remove the sucrose used in producing the density gradient. Improvement of technique may remedy this factor. It must be recognized that as purification progresses, one might expect low recovery due to possible synergistic action of the various components. This is conjecture and would have to be proved by mixing pure fractions.

TABLE III.

Column electrophoretic analysis of Pool J. Phosphate buffer, pH *7.7, ionic strength, 0.01, and a sucros gradient were used. Current was 15 m.a.*

Pool	500 mg sample				300 mg sample			
	Dry wt. mg	HU/mg	TU/mg	HU/TU	Dry wt. mg	HU/mg	TU/mg	HU/TU
CE-1	1,004.4 *)	0	0		0	0	0	
CE-2	182.0	0	0		8.8	0	0	
CE-3	38.9	<1	0.5		7.0	0.5	2.0	
CE-4	126.7	30	3.0	10	44.0	25.0	5.0	5
CE-5	214.6	70	7.0	10	87.1	125.0	12.5	10
CE-6	136.3	0	0		4.0	0.5	0.5	1
CE-7	14.7	0	0		0	0	0	

*) Mostly sucrose because of incomplete dialysis.

The toxin apparently is protein in character. Some evidence has been furnished that the hemolytic and toxic factors are separate (TILDEN et al., 1961) since the toxic factor seems more stable to heat than does the hemolytic factor. In addition, the HU/TU ratio changes with acetone purification. From electrophoretic analysis, however, it would seem that the hemolytic and the toxic factors are rather closely associated during migration in an electric field. By re-running samples, greater resolution may separate what are two rather close electrophoretic components. The toxic factor and the hemolytic factor may be two separate proteins or protein complexes having similar charge properties. Another possibility is that toxic and hemolytic activity are separate factors exhibiting synergism. A third possibility is that the toxin is a complex molecule which has both hemolytic and toxic properties. Alteration of the molecule could affect one or both of the activities. The hemolytic component may also be toxic, but additional toxic components are present in the crude preparations. As purification proceeds, one may be dealing more and more with the hemolytic component. Greater purification will be necessary to elucidate these interesting possibilities.

SUMMARY

The endotoxin from *Aspergillus fumigatus* was studied and appears to be protein in character. Attempts to purify the material by fractional precipitation with acetone resulted in increase of hemolytic and toxic potencies. The hemolytic activity of the toxin is stable from pH 3.6 to 8.4.

Electrophoretic analysis enabled the assignment of hemolytic activity to a definite electrophoretic component.

Chromatography with DEAE cellulose using gradient elution effected increase in hemolytic activity. The bioassay for toxic activity is less quantitative than the assay for the hemolytic activity.

Column electrophoresis confirmed the assignment of hemolytic potency to an electrophoretic component. Recovery of activity was low in this method.

Both the hemolytic and toxic activities occur in the same electrophoretic component; however, there is evidence that they may be separate components.

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SUR LA PRÉSENCE DU TRICHOPHYTON (ACHORION) QUINCKEANUM DANS LE SOL

par

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(21.XI.1960)

(avec 3 figs.)

Le *Trichophyton* (*Achorion*) *quinckeanum*, dermatophyte vivace, est assez répandu à Bucarest et dans notre pays. Chez nous, sa présence a été signalée, non seulement dans des lésions mycosiques de la souris ou du chat, mais souvent aussi chez l'homme. Ce champignon, dont les caractères botaniques ne diffèrent essentiellement (4) de ceux propres au *Ctenomyces interdigitalis* (*Epidermophyton* Kaufmann-Wolf) et au *Ctenomyces mentagrophytes* (*Trichophyton gypseum*) n'a pas été isolé exclusivement des lésions à godets. Chez l'homme il est capable de provoquer une foule de dermatomycoses. D'ailleurs, dans un travail présenté à l'onzième Congrès International de Dermatologie, de Stockholm (août 1957), par Nicolau, par l'un de nous (Evolceanu) et par Avram (11), on avait démontré que ce dermatophyte est capable de provoquer les dermatomycoses les plus diverses telles que le favus de la peau glabre et du cuir chevelu, le herpès circiné simple ou inflammatoire, des trichophyties plus ou moins inflammatoires du cuir chevelu ou de la barbe et, parfois, des vrais kérions. D'après les auteurs suscités, l'*Achorion quinckeanum* serait identique au *Trichophyton niveum* de Sabouraud. Depuis lors, les faits observés par nous confirmèrent cette opinion car, inoculé à la souris blanche, le soit-disant *Trichophyton niveum*, qu'il fût isolé d'une lésion sèche ou inflammatoire (kérion), nous a conduit à un même résultat: l'apparition des godets faviques caractéristiques [1]).

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[1]) Chez le *Trichophyton quinckeanum* nous avons toujours trouvé des aleuries nombreuses, en acladium et en grappes. Quand il y avait des fuseaux, ceux-ci étaient pareils à ceux des *Ctenomyces* et pas des *Microsporons*. Par conséquent, l'opinion de Conant et col. (4), selon lesquels le *Trichophyton quinckeanum* ne se distingue guère des microïdes de Sabouraud, nous la considérons plus plausible que la thèse de Langeron, qui place ce champignon dans le genre *Sabouraudites*, à côté des *Microsporons*. (9).

Très proche au *Ctenomyces interdigitalis* et au *Ctenomyces mentagrophytes*, le *Trichophyton quinckeanum* est, bien sûr, comme ceux-ci, une espèce primitive, pas encore complètement adaptée à la vie parasitaire. Sa riche morphologie microscopique, de même que le caractère vivace de ses cultures et l'allure inflammatoire des lésions qu'il provoque, ainsi que la rareté relative de sa présence comme parasite, en sont autant d'arguments. Si les espèces les plus primitives des dermatophytes, à savoir le *Microsporon gypseum* et le *Keratinomyces ajelloi*, sont en réalité de simples saprophytes du sol, il va sans dire que les autres dermatophytes primitifs, tels que les *Ctenomyces* et le *Trichophyton quinckeanum* etc. devraient aussi y être présents. En ce qui concerne le *Ctenomyces mentagrophytes*, ce champignon a été isolé, en 1955, par LURIE & BOROK, du sol d'une grotte en Afrique (10). Antérieurement à ces auteurs, le dermatomycologue hongrois SZATHMÁRY paraît l'avoir isolé de la vase de certaines rivières (12). En 1959, AJELLO (1) a trouvé le *Microsporon cookei* dans les couches superficielles du sol et, tout récemment, EVOLCEANU, ALTERAS et col. y ont mis en évidence le *Ctenomyces interdigitalis* (8). Quant à l'*Achorion quinckeanum*, sa présence dans le sol n'avait pu être démontrée qu'indirectement. En 1957, VANBREUSEGHEM publie l'observation d'un petit malade, âgé de 6½ ans, qui s'est traumatisé le genou en tombant et chez lequel il s'est développé, par la suite, sur la région lésée, un herpès circiné typique. De cette lésion on a isolé le *Trichophyton quinckeanum*, dermatophyte que l'on n'a pas, malheureusement, réussi à mettre en évidence dans la terre du jardin où jouait l'enfant (14). D'ailleurs il paraît logique d'admettre que le *Trichophyton quinckeanum* soit présent dans le sol. L'hôte de ce dermatophyte est surtout la souris, animal très attaché au sol par son mode d'existence. L'observation de VANBREUSEGHEM vient de rendre plus vraisemblable cette supposition.

Les considérations sus-mentionnées nous ont conduit de chercher systhématiquement le *Trichophyton quinckeanum* dans les portions du sol qu'on pouvait soupçonner être source de contamination des mycoses observées par nous et provoquées par ce parasite. Ces recherches ont confirmé nos suppositions.

Rapportons ici les faits observés par nous:

Le 29 février dernier (1960) on nous a envoyé une malade âgée de 32 ans, polisseuse de meubles, atteinte depuis 2—3 semaines d'une dermatose, que l'on soupçonnait être une mycose. A l'examen, la face antérieure du poignet gauche était occupée par une plaque d'herpès circiné dont le diamètre mesurait 2½ cm environ. Le pourtour en était marqué par une couronne de petites vésicules qui, vers le centre de la plaque, faisaient place à des petites érosions confluentes, formant une bande large de 3 mm. Le centre de la lésion était occupé par des croûtes et des cicatrices. Des godets ne s'y trouvaient pas.

Au dire de notre malade, son affection s'était manifestée, au début, par des phénomènes inflammatoires assez intenses. Quand elle s'est présentée à nous, l'inflammation avait déjà beaucoup diminué. Toutefois, il y avait encore une certaine infiltration locale. La lésion s'est montrée assez rebelle au traitement et la guérison n'a pu être obtenue que par de nombreuses applications locales d'une pomade au calomel 30 %. L'examen microscopique des vésicules périphériques confirma le diagnostic de mycose et les cultures montraient qu'il s'agissait du *Trichophyton quinckeanum.*

La malade n'ayant pu préciser l'origine de son infection, nous nous transportâmes à son domicile où nous constatâmes, dans la cour, l'existence d'un petit abri pour le bois. Au dire des locataires, cet abri était fréquenté par des souris ou par des rats, et supposant qu'elle s'était contaminée là, en fendant le bois, nous y récoltâmes une certaine quantité de terre. En utilisant le procédé de VANBREUSEGHEM (13,5), nous réussîmes très vite à mettre en évidence, dans cette terre, la présence du *Microsporon gypseum* et du *Keratinomyces ajelloi.* Ces derniers dermatophytes, étant extrêmement répandus chez nous dans le sol, leur présence dans la terre examinée n'a pas retenu notre attention. Il n'en était pas de même de 3 autres espèces de dermatophytes, que nous avons découvert dans cette terre, à savoir le *Trychophyton terrestre* DURIE & FREY et, de plus, le *Trichophyton quinckeanum.* La description du *Tr. terrestre* fera l'objet d'un travail ultérieur. L'isolement de ce dernier et surtout celui du *Trichophyton quinckeanum* a été bien plus difficile à réaliser que celui du *Microsporon gypseum* et celui du *Keratinomyces ajelloi.* Les cheveux, préalablement stérilisés, mis en contact avec la terre (conformément au procédé de VANBREUSEGHEM), il nous a fallu les contrôler systhématiquement pendant plus de deux mois, avant de reconnaitre, à leur surface, les petites aleuries sphériques ou ovalaires, propres aux dermatophytes. En ensemençant ces dernières sur milieu de Sabouraud, nous avons réussi, après plusieurs passages, à isoler les dermatophytes sus-mentionnés.

ÉTUDE MYCOLOGIQUE DU TRICHOPHYTON QUINCKEANUM ISOLÉ DE LA TERRE.

Sur milieu d'épreuve de Sabouraud, l'aspect des cultures était le même que celui des cultures du parasite isolé de la malade sus-mentionnée, à savoir l'aspect propre aux cultures du *Trichophyton quinckeanum.* La partie centrale en était légèrement duveteuse, parfois sillonnée (dans les cultures d'un certain âge) par des plis rayonnants, peu profonds, tandis que la partie périphérique prenait un aspect presque poudreux (fig. 1) La couleur des cultures restait invariablement blanche.

D'après les observations de certains auteurs (3) et d'après nos observations personnellles, les cultures du *Trichophyton quinckeanum,* quand elles ont atteint un certain âge, deviennent souvent plus ou moins roses, en même temps que leur face profonde devient

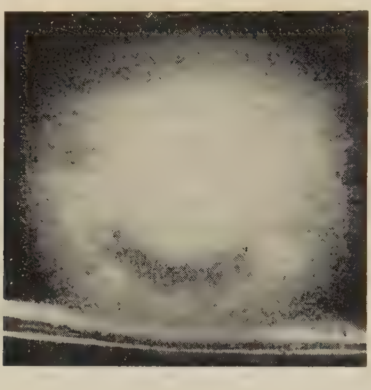

Fig. 1. *Trichophyton quinckeanum.* Culture de 20 jours, sur agar glucosé de Sabouraud, en tube (souche isolée de la terre).

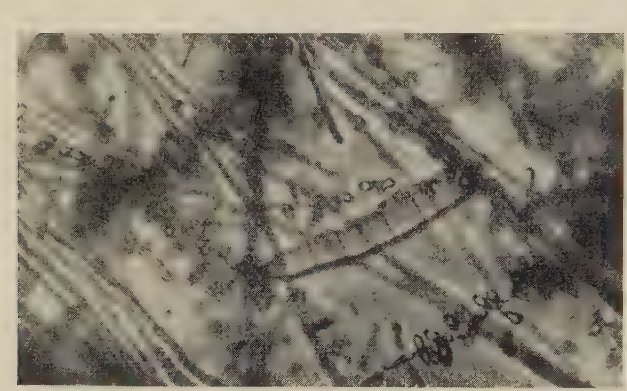

Fig. 2. *Trichophyton quinckeanum.* (souche isolée de la terre). Aleuries at macroconidies (culture de 20 jours, en goutte pendante, sur milieu de Sabouraud).

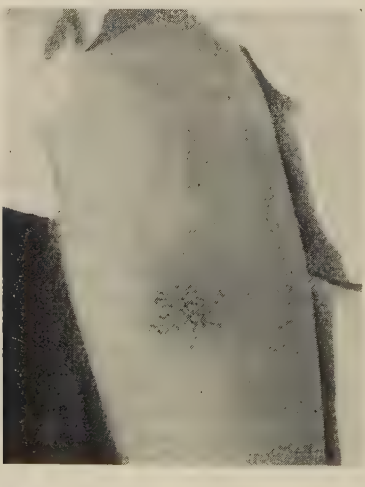

Fig. 3. Mycose expérimentale de l'homme, due au *Trichophyton quinckeanum* (souche isolée de la terre); godets faviques (avant-bras).

violette. Toutefois, les cultures dont il s'agit ici n'ont produit aucun pigment. Le pigment était d'ailleurs absent aussi dans les cultures du champignon isolé de la malade. Leur face profonde en était aussi dépourvue. L'aspect des cultures maintenues à la température de la chambre était le même que celui des cultures maintenues au thermostat (à 37° C).

Les microcultures en goutte pendante montraient des aleuries disposées en acladium et en grappes, ainsi que d'assez nombreux fuseaux plutôt en quenouille, septés et dont l'extrémité libre était légèrement arrondie (fig. 2).

INOCULATIONS EXPÉRIMENTALES DU CHAMPIGNON ISOLÉ DE LA TERRE

Inoculations à l'homme.

Deux personnes seulement, plus précisément deux femmes, âgées respectivement de 30 et de 50 ans, ont été inoculées et, dans chacun de ces deux cas, on a obtenu un résultat positif. L'inoculation a été pratiquée à la face antérieure de l'avantbras. Elle a été suivie, au bout d'une semaine environ, par l'apparition d'une tache rouge, congestive, laquelle est devenue, les jours suivants, légèrement squameuse. Tandis que chez la femme la plus jeune les choses en sont restées là, chez la femme la plus âgée on a observé l'apparition, sur cette tache, de nombreuses petites saillies acuminées qui prenaient, peu à peu, l'aspect des vrais godets faviques, dont le diamètre mesurait 1-2 mm (fig. 3). Chez la personne la plus jeune, on a pu constater seulement la présence des godets microscopiques. D'ailleurs, chez elle, la lésion a complètement guéri, au bout de 10-12 jours, sans aucun traitement. Par contre, chez la femme la plus âgée, la lésion a persisté plus d'un mois, malgré les traitements employés.

Inoculation à l'animal.

Nous avons inoculé 4 souris blanches et 3 cobayes. L'inoculation a été pratiquée sur le dos des animaux, en utilisant le procédé habituel. Les cultures, triturées à l'aide du papier de verre, ont été déposées sur la peau de ces animaux, après l'épilation de la région qui devait être inoculée et après avoir frotté cette région à l'aide du même papier. Voici les résultats: Chez deux des souris et chez deux des cobayes inoculés, on a observé, au bout d'une huitaine de jours, l'apparition d'une petite plaque squameuse. Il n'y avaient pas de godets visibles. Les squames étaient riches en filaments mycéliens qui y persistèrent pendant plus d'une semaine. Chez les cobayes les poils avaient été partiellement envahis, la disposition du parasite revêtant l'aspect ecto-endothrix. Chez les souris, les poils n'ont pas été envahis mais on y observait des godets microscopiques.

L'ensemencement du matériel pathologique (vésicules, squames, godets), récolté des lésions expérimentales de l'homme et de l'animal, a reproduit exactement la souche inoculée.

DISCUSSIONS

Le fait d'avoir isolé le *Trichophyton quinckeanum* du sol, à l'endroit même où notre malade fendait le bois, vient à l'appui de l'hypothèse d'après laquelle son infection a eu une origine tellurique. En effet, il est bien probable que la souche, isolée par nous de la terre, soit la même que celle isolée de la malade. L'identité de ces deux souches est rendue plus vraisemblable par la ressemblance qui existe entre elles, tant du point de vue de l'aspect macroscopique (absence du pigment) que du point de vue de celui microscopique (fréquence relative des macroconidies) de leurs cultures. Cette ressemblance n'est pas fortuite, car chez le *Trichophyton quinckeanum* les caractères sus-mentionnés se rencontrent plus rarement. On trouve souvent des souches chez lesquelles les macroconidies sont très peu nombreuses ou absentes ainsi que des souches pigmentées.

On peut se demander si cette souche n'avait pas été déposée sur le sol par des souris atteintes de favus, mais on peut tout aussi bien se demander si au moins une partie des souris malades ne se seraient infectées par le sol où le *Trichophyton quinckeanum* vivrait comme saprophyte [1]). En effet, on pourrait admettre que le *Trichophyton quinckeanum* mène une partie de son existence comme saprophyte du sol ou qu'il soit, par sa nature même, plutôt saprophyte du sol que parasite, c'est à dire que le parasitisme manifesté par lui soit le phénomène second. D'autre part, d'après ce qu'il résulte de nos inoculations expérimentales, la souche isolée par nous de la terre s'est montrée un peu moins pathogène envers la souris qu'envers l'homme. Ce fait rend l'origine murine de notre souche peu probable et vient plutôt à l'appui de son origine tellurique.

La terre récoltée par nous montrait cette fois une flore dermatophytique assez complexe. Outre le *Microsporon gypseum*, le *Keratinomyces ajelloi* et le *Trichophyton quinckeanum*, nous y avons isolé, comme nous l'avons déjà dit plus haut, le *Trichoph. terrestre*, proche voisin au *Ctenomyces mentagrophytes* mais pas identiques à lui. Le *Trich. terrestre* ne saurait pas provenir de la souris, car il s'est montré très peu pathogène envers cet animal.

Nous pensons que le *Trichophyton quinckeanum* et le *T. terrestre* déjà mentionné doivent être assez répandus dans les couches superficielles du sol. Malheureusement, on ne connaît, jusqu'à présent, un moyen assez efficace, capable de toujours isoler du sol, quand il s'y trouvent, ces dermatophytes et les séparer des autres champignons plus keratinophyles (surtout du *Microsporon gypseum*), très répandus dans ce milieu naturel. Nos recherches se poursuivent.

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[1]) Ce même mécanisme pathogénique pourrait être invoqué aussi pour expliquer, au moins dans certains cas, l'apparition, chez les animaux, d'autre mycoses soit-disant spontanées, par exemple celles provoquées par le *Ctenomyces mentagrophytes*, par le *Microsporon gypseum*, par le *Microsporon lanosum* et par d'autres dermatophytes vivaces.

Il serait utile et même nécessaire de continuer les investigations dans cette voie car, si l'on a pu démontrer que deux dermatophytes vivaces et à riche morphologie microscopique, à savoir le *Microsporon gypseum* et le *Keratinomyces ajelloi*, sont des simples saprophytes du sol, en voie d'adaptation à la vie parasitaire, il est très probable que d'autres dermatophytes vivaces reconnaissent la même origine [1]). Ce raisonnement nous le considérons valable même si nous admettons que ces autres dermatophytes fussent quelque peu plus avancés dans cette voie d'adaptation à la vie parasitaire, adaptation que l'on ne peut pas considérer complète, vu le caractère vivace de leurs cultures et leur riche morphologie microscopique.

Résumé

En continuant leurs recherches, en vue de contribuer à l'élucidation du problème concernant la nature saprophytique, tellurique, des dermatophytes, les auteurs ont réussi, en utilisant le procédé de VANBREUSEGHEM, à isoler du sol le *Trichophyton quinckeanum.* Ils l'ont mis en évidence dans la terre qui se trouvait sous un petit abri pour le bois, abri situé près d'une maison à Bucarest. La même souche avait provoqué un herpès circiné inflammatoire chez une femme qui s'est probablement contaminée en fendant le bois sous ce même abri. La souche isolée de la terre a été inoculée, avec résultat positif, à l'homme, au cobaye et à la souris blanche. Chez l'homme, la mycose expérimentale a été accompagnée par l'apparition de godets visibles à l'oeil nu (dans un seul cas).

Selon les auteurs, il serait utile de chercher systhématiquement, dans les couches superficielles du sol, tous les dermatophytes à cultures vivaces et à riche morphologie microscopique, vu qu'ils ont isolé, de la même terre et en même temps que le *Trichophyton quinckeanum* (et en même temps que le *Microsporon gypseum* et le *Keratinomyces ajelloi*) le *Trichophytron terrestre* proche au *Ctenomyces mentagrophytes.*

Summary

In continuing their researches in order to contribute to the elucidation of the problem concerning the presence of dermatophytes as saprophytes in the soil, the authors were able to isolate from the soil, making use of VANBREUSEGHEM's method, a strain of *Trichophyton* (*Achorion*) *quinckeanum.* The very difficult isolation of this fungus has been done from the soil of a woodshed, in a court of a house in Bucarest. The same strain caused a herpes circinatus of inflammatory type, on the wrist of the woman to whom the shed belonged. This woman probably contaminated herself by

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[1]) Récemment nous avons réussi à mettre en évidence, dans une petite quantité de terre, qu'on nous a apporté de Vietnam, le *Trichophytron terrestre* sus-mentionné.

spliting wood there. The strain, isolated from the soil, was inoculated in man, guinea pig and white mouse, with positive results. In one case, the experimental lesion, obtained in man, showed the presence of little scutula. Considering that lately they were able to isolate from the soil, not only the above mentioned *Trichophyton quinckeanum* (in addition to *Microsporon gypseum* and *Keratinomyces ajelloi*) but also the *Trichophytron terrestre*, near to *Ctenomyces mentagrophytes*, the authors think that it would be useful to persist in searching farther, in the superficial layers of the soil, for the presence of the dermatophytes with rich microscopical morphology.

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Moore, W. C., British Parasitic Fungi. A Host-parasite Index and Guide to British Literature on the Fungus Diseases of Cultivated Plants. Cambridge, University Press, 1959. xvi and 430 pages. 45 s.

Moore's "British Parasitic Fungi" is a comprehensive and conveniently arranged book which authoritatively covers the subject defined in the sub-title. The introductory portion includes a list of source books and lists of abbreviations of recurring place names and selected references.

Part I of the text is an alphabetical list of generic (in some instances, specific) and cross-indexed common names of host plants. The recorded parasitic fungi of each host are listed alphabetically under the scientific name of the host.

Part II alphabetically lists the parasitic fungi from *Acanthostigma parasiticum* to *Zythia fragariae.* The name of the fungus is followed by a citation to its first description, its listing in Saccardo's "Sylloge Fungorum" and references to important papers describing the fungus, the disease and control of the disease. The list includes many names which are synonyms. These, and the names of Imperfects (where applicable) are cross-indexed to the correct name. Of three binomials included under *Cercospora,* two are referred to other genera. In view of experimental studies, published elsewhere, which report successful cross inoculations, the validity of some of the 16 listed species of *Cercospora* may be questioned. However, plant pathologists commonly recognize several hundred species of *Cercospora.* Sound criteria for determining synonymy within this taxonomic morass await additional experimental studies.

The annotations accompanying most of the listings are very valuable features of the book. The notes include information about historical aspects of some diseases, resistant varieties of host species, pertinent aspects of epidemiology and methods of control. Under some important pathogens the more extensive annotations are conveniently subdivided. *Phytophthora infestans and Botrytis cinerea* rate 8 pages each. *Corticium solani*, *Venturia inequalis*, *Verticillium albo-atrum* and the edible but very destructive Basidiomycete, *Armillaria mellea* require 4—5 pages each.

An interesting feature of the book is the inclusion of several troublesome slime molds, actinomycetes (often included among the bacterial causes of plant diseases) and some of the fungi which parasitize or are weeds of commercial mushroom beds. The medical mycologist (if not already informed about plant pathology) may be impressed by the much greater relative importance of fungi in the etiology of diseases of plants compared with the clinical importance of fungal versus bacterial diseases of man

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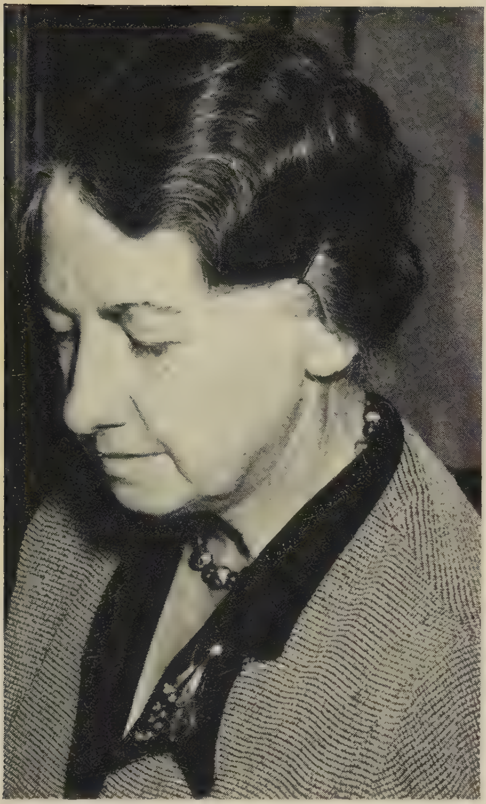
Milestones in Microbiology. Translated and edited by THOMAS D. BROCK. Paperback. 275 pp. Prentice-Hall, Inc. Englewood Cliffs, N.J. U.S.A. $ 3.95.

This work is not a history of microbiology. It is a collection of papers spanning the time from 1677 (VAN LEEUWENHOEK) to 1924 (A. J. KLUYVER) in six Parts. Part I deals with Spontaneous Generation and Fermentation. Part II brings The Germ Theory of Disease. Part III brings papers on Immunology. Part IV deals with Virology. Part V discusses Chemotherapy. Part VI deals with General Microbiology.

There are no papers on fungi, algae, or protozoa in this collection. The whole has a definite medical orientation, since the nineteenth century emphasis was primarily medical. Yet, however vast a field microbiology represents today, mycologists, particularly medical mycologists, will enlarge their horizon by reading these basic papers, every one of which added a milestone on the arduous way of development of the knowledge which microbiology encompasses today. The papers are well selected. The translation is impeccable. The production of the book is satisfactory. It is highly recommended to mycologists, bacteriologists and to all those who have interest in and understanding for the historical evolution of this special branch of science.

TIBOR BENEDEK



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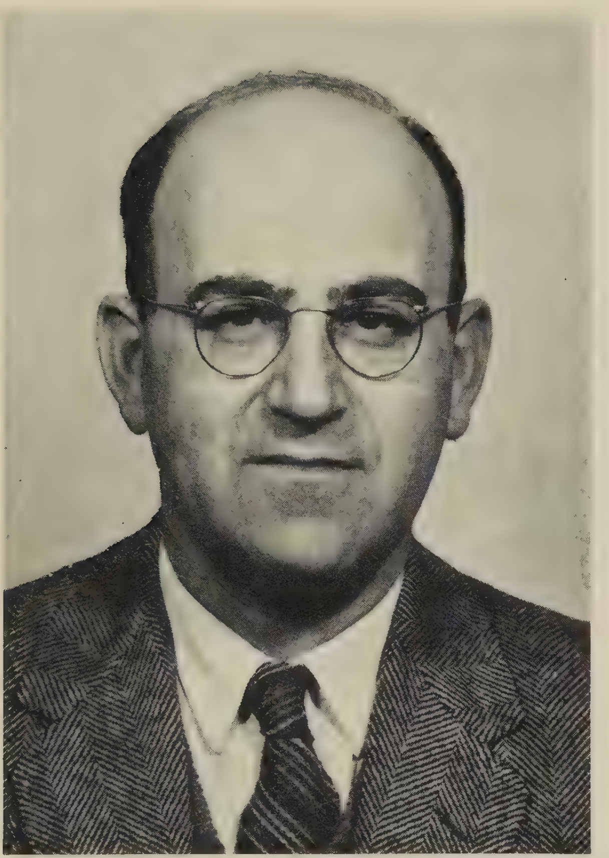

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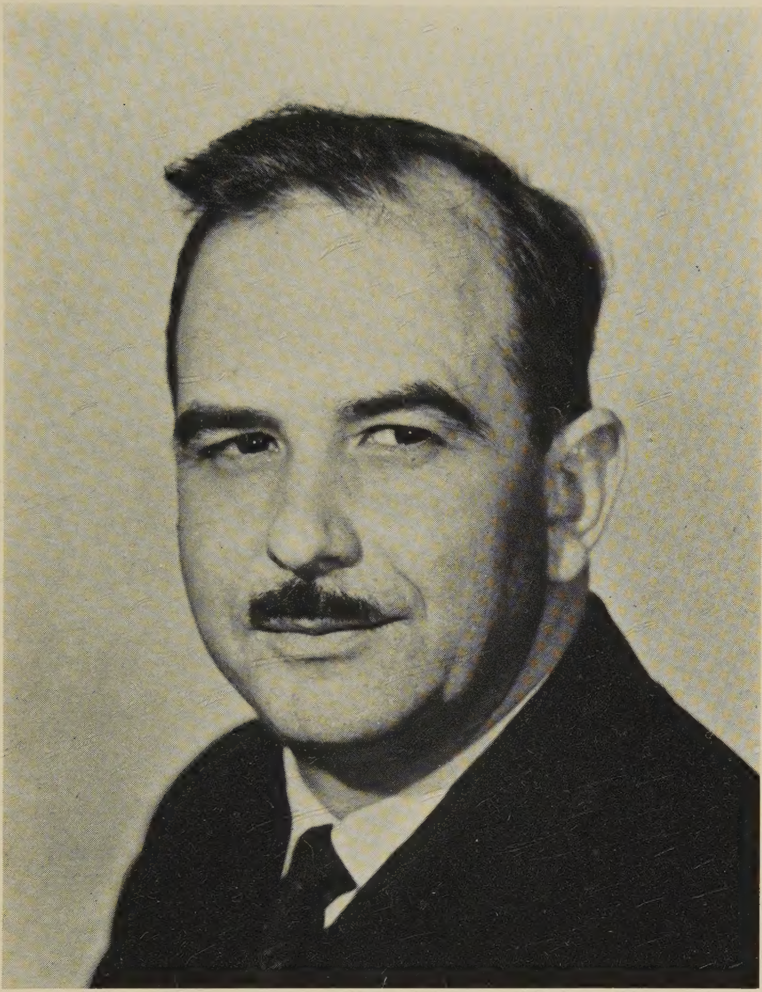

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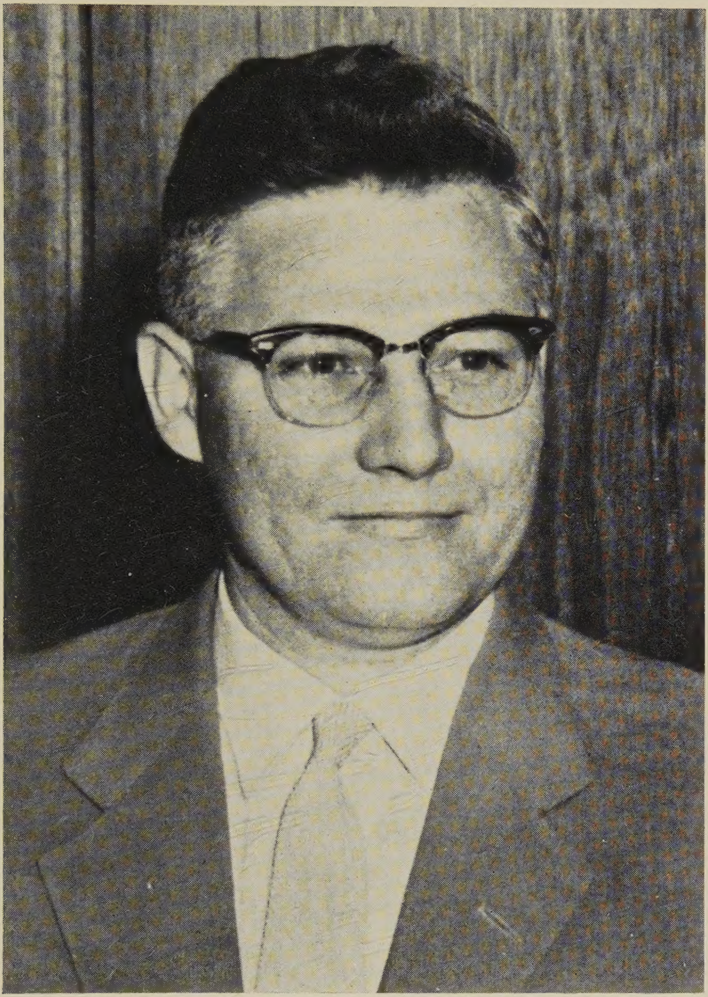

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